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ORIGINAL ARTICLES

THE RELATION OF THE COMMON BILE DUCT TO THE PANCREATIC DUCT IN COMMON DOMESTIC AND LABORATORY ANIMALS

BY F. C. MANN, M.D.; J. P. FOSTER, V.M.D.; AND S. D. BRIMHALL, V.M.D.
ROCHESTER, MINN.

THE relation of the common bile duct to the pancreatic duct is of some importance from the physiologic standpoint and of great importance from the pathologic standpoint. In studying the function of the gall bladder it seemed desirable to know whether there is any particular relationship of the common bile duct to the pancreatic duct in species of animals with a gall bladder as compared with species without a gall bladder.

There have been a few general and some special studies made on the relationship of these two ducts.[2, 3, 4, 6, 8, 9, 10, 11] No attempt seems to have been made to obtain data with regard to the function of the gall bladder or the cause of pancreatitis by a study of this phase of comparative anatomy.

We made a comparative study of the relationship of these two ducts in species of animals that can be used for research purposes. A few species not commonly employed in laboratories were added for comparison. In each species several dissections were made, and both fresh and fixed material were employed. An attempt was made to group the species with reference to the relationship of the two ducts. On this basis all the species studied can be included in three groups.

Group 1.—This is the simplest group and contains the species in which both ducts enter the duodenum by separate openings. The animals investigated in this group were the ox, the pig, the rabbit,* the guinea pig, and the striped gopher (C. tridemlineatus).

*This species, as well as some of the others in Group 1 might have been included in Group 2, but as a functionating duct is very rarely found entering with the bile duct, the species was placed in Group 1.

Group 2.—This group, to which man belongs, is probably the best known; it contains those species in which the two ducts are distinct, but have a common entrance into the duodenum. Each species in this group, either regularly or occasionally, has a second or accessory pancreatic duct opening at some distance

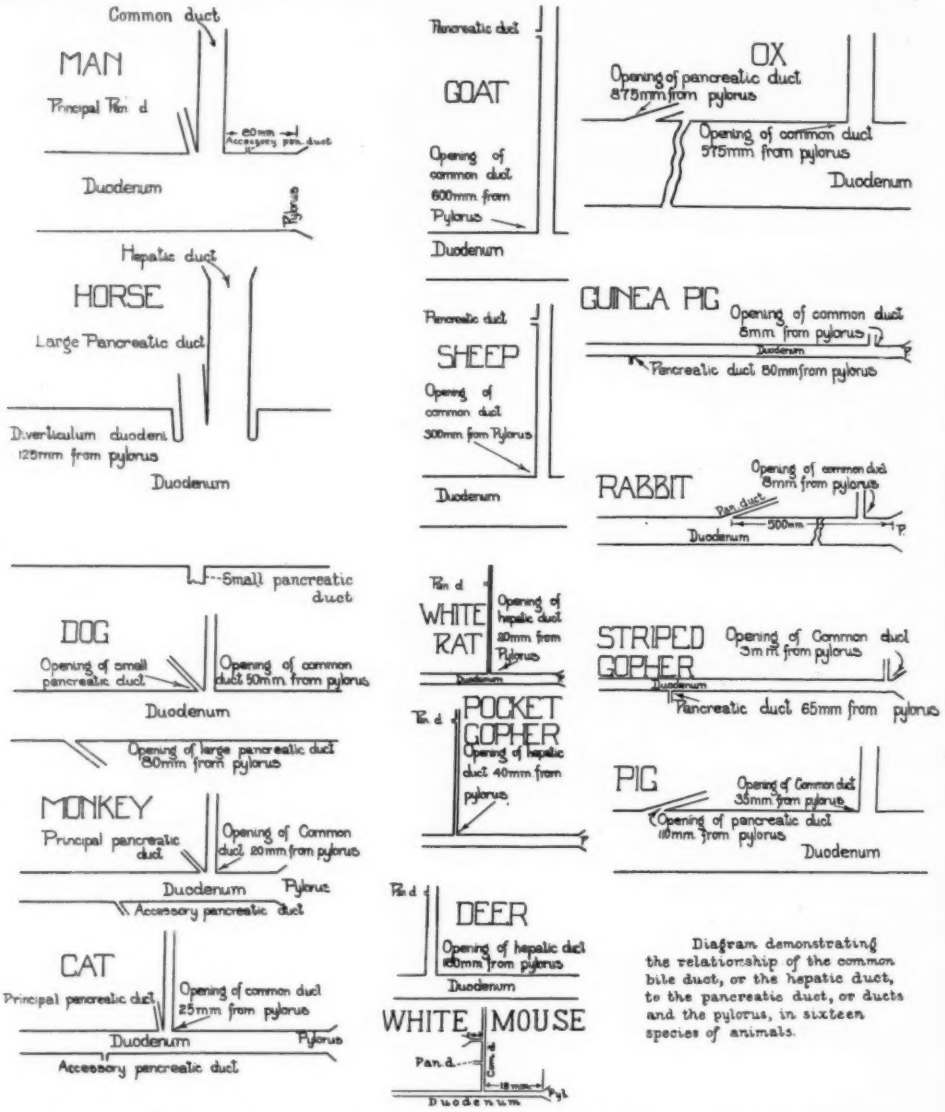

Fig. 1.—Diagram showing the exact size and relations of the bile duct to the pancreatic duct and the pylorus. The column on the left hand side contains those species included in Group 2; the column on the right side contains those species included in Group 1, while in the middle column are the species in Group 3.

from the common opening of the two ducts; in some instances this second duct is the major one. The species examined in this group were the horse, the monkey (M. rhesus), the dog, and the cat.

Group 3.—In this interesting group are the species in which the pancreatic duct empties directly into the common bile duct, usually at quite a distance from

the opening of the latter into the duodenum. The animals studied in this group were the goat, the sheep, the deer, the mouse, the rat, and the pocket gopher (G. bursarius).

We have not been able to find any reason why the various species should fall into these groups when they are classified on the basis of the relationship of the two ducts. No other common factor was found in the species in any of the groups. Some very general statements may, however, be made:

The majority of the species noted in Group 1 belong to the rodentia and have some general traits in common. No closer comparison can be made. The only carnivora studied fall in Group 2, but animals of the group as a whole have nothing in common. In Group 3 the goat, the sheep, and the deer are closely related in some of their life habits, but have nothing in common with the remaining species of that group.

The important points to consider in the relationship of the comparative anatomy of these ducts to their comparative physiology and pathology are: (1) the presence or absence of a gall bladder, (2) the relation of the point of entrance of the duct to the pylorus, and (3) the presence or absence of a tendency to develop pancreatitis.

Four of the species studied and grouped in our classification do not possess a gall bladder. One of these, the horse, belongs to Group 2, and the other three, the deer, the rat, and the pocket gopher belong to Group 3. All the species included in Group 1 have a gall bladder. It is quite possible that if a larger number of species had been studied, one without a gall bladder, in which the two ducts open separately, might have been found. We have not been able to find mention of such a species; the nearest approach to this was in the horse, in which the ducts are quite distinct but have a common opening. It is interesting to note that in most of the species in which a gall bladder is absent, the pancreatic duct empties directly into the common bile duct. Probably much importance should not be attached to this, however, because other species in which the gall bladder is well developed also have the same anatomic structure. (Compare the deer, goat, and sheep).

One of the most interesting interactions in the body is that between acid and alkali at the pylorus. From the standpoint of comparative anatomy the important point is a consideration of the relation of the pylorus to the entrance of the biliary and pancreatic ducts. A review of our data shows that the species can not be grouped with reference to this relationship.

No comprehensive comparative study of pancreatitis seems ever to have been made; our knowledge of the disease has been secured by the study of the condition in man. The usual explanation for the cause of pancreatitis in man, particularly of the acute form, is that it is due to the injection of bile into the pancreatic duct following the pathologic transformation of the two ducts into a closed system.[1, 7] According to this hypothesis one would anticipate, from our data on the comparative anatomy of the two ducts, that some species of animals (those of Group 3 that possess a gall bladder) would have pancreatitis quite frequently, and that other species (those in Group 1) would very rarely have this disease. We have been unable to obtain any data to substantiate this. The

extensive experiences of two of us (Foster and Brimhall) in inspecting carcases in the packing plants show that pancreatitis in the species observed and at the ages at which the animals are slaughtered is exceedingly rare. The experiences of one of us (Mann) with pancreatitis in laboratory animals are identical with those of Foster and Brimhall. The results of a large series of necropsies on dogs, goats, cats, rabbits, and guinea pigs show that pancreatitis must be very rare in these species. In our laboratory, spontaneous pancreatitis has been observed only in dogs, and but in a very few instances in this species.[5] These data seem to show that pancreatitis is not common in the species of animals included in our study, or that it is not present at the ages at which the animals come to necropsy. Also there does not seem to be a relationship of the comparative anatomy of the biliary and pancreatic ducts to pancreatitis.

SUMMARY

A comparative study of the relationships of the common bile duct to the pancreatic duct was made in fifteen species, which represented mainly the common domestic and laboratory animals. It was found that each of the species could be included in one of three groups. No reasons for the relationship of the two ducts in the different groups were found. It was noted that there was no definite connection between the presence or absence of the gall bladder and the relationship of the two ducts, although of the four species in the series of animals that did not possess a gall bladder, three belonged to one group.

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A SECOND MODEL ILLUSTRATING SOME PHASES OF KIDNEY SECRETION*

By Martin H. Fischer, M.D., Cincinnati, Ohio

I. INTRODUCTION

I HAVE recently made use of a principle first discovered by Thomas Graham and of another emphasized chiefly by Maly which, in connection with various observations of my own, make easy the laboratory demonstration of certain physiologic and pathologic facts regarding secretion in general, or urinary secretion in particular, which are ordinarily assumed to be inexplicable in simple physicochemical terms, and, for the understanding of which, the vague, "vital" forces of protoplasm are too often called into action. It is still a source of wonder to many biologic workers that a neutral or alkaline kidney parenchyma can be the mother of an acid urine; that the kidney assiduously "protects" the living animal from being overwhelmed by acid or alkali; that various constituents characteristic of urine are found here in a different proportion from that of these same substances in the kidney parenchyma or in the blood flowing through this; etc. It is to the further analysis of the nature of these allegedly strange biologic facts and to their easy demonstration in class room that the remarks of this paper are addressed. As the following shows, the entire biologic fabric is readily reproducible from the simple strands furnished by the concepts of modern colloid chemistry.

II. NOTES ON GRAHAM'S DIALYSIS EXPERIMENTS

Thomas Graham[1] showed many years ago that when any readily hydrolyzable salt, like ferric chloride, is put into a sac (like parchment) which is permeable to molecularly dispersed substances (crystalloids), but impermeable to more grossly dispersed ones (colloids) and the whole is then hung into water, as shown in Fig. 1 (is subjected, in other words, to dialysis) the following changes occur: Because of the hydrolysis of the ferric chloride, ferric hydroxide and hydrochloric acid are produced. Since the hydrochloric acid is highly diffusible, it at once begins to escape through the parchment membrane into the surrounding bath of water. Ferric hydroxide remains behind.

The example here chosen is, of course, not an isolated one. It is characteristic in general of all the hydrolyzable salts. This dialysis method was, in fact, used by Graham for the production of many different kinds of nondiffusing "colloids" from the pure solutions of what were originally only molecularly dispersed and highly diffusible salts.

A quantitative study of the *chemical* changes which occur in the experiment just described has been made by S. E. Linder and H. Picton.[2] This shows that the proportion of iron to hydrochloric acid steadily increases within the diffusion thimble while it as steadily decreases in the water surrounding the thimble. In other words, the amount of ferric hydroxide increases progressively within the

*From the Eichberg Laboratory of Physiology in the University of Cincinnati.

thimble, while the amount of the hydrochloric acid increases without. Iron finally ceases to come out from the internal liquid. The reason for all this is found in the fact that the originally diffusible iron chloride gradually goes over into a "colloid" iron hydroxide which can no longer pass through the parchment membrane since it contains no holes large enough to let such supermolecular aggregates through.

A quantitative study of the *physical* changes of the thimble contents in this dialysis experiment has been made by N. Sahlbom.[3] By subjecting the solution of iron contained within the diffusion capsule to "capillary analysis" (in which are utilized the principles first discovered by John Uri Lloyd[4] and Friedrich Goppelsroeder[5]) the gradual replacement of the originally highly diffusible iron salt by a nondiffusing colloid one can easily be made a matter of ocular demonstration. If strips of filter paper are simply dipped into the liquid contents of the diffusion thimble on successive days, it is found that, on the first day, both the dispersion medium (water) and the dispersed substance (iron salt) ascend the paper as shown in the first strip of Fig. 2. However, as the change in the thimble contents progresses, the dispersion medium is still found to ascend the paper, but there is a progressive reduction in the height to which the iron salt will go. In the final stages of the experiment (after the dialysis has been continued for several days) the colloid ferric hydroxide scarcely diffuses at all, coming to rest almost immediately above the surface of the liquid into which the filter paper has been dipped, as shown in the right hand strip in Fig. 2.

Fig. 1.

Fig. 2.

This whole experiment may be readily repeated for demonstration purposes in the following fashion: An ordinary parchment paper diffusion thimble (one capable of holding 20 c.c. or more) is filled to near its top with a ⅕ molar (about 5 per cent) solution of ferric chloride ($FeCl_3.6\ H_2O$). The thimble with its contents is then suspended in a larger bottle or Erlenmeyer flask as shown in Fig. 1, enough distilled water being poured into the container to bring its level up to the level of the ferric chloride in the thimble. The distilled water must, naturally, be changed two or more times daily in order to get rid of the products of diffusion.

The acid nature of the wash water is readily demonstrated by adding to it some indicator like methyl red. The presence of iron in it is betrayed by the slightly yellowish tinge observable in the first few hours or days of the experiment. If desired, the iron may be demonstrated quantitatively by adding to a given

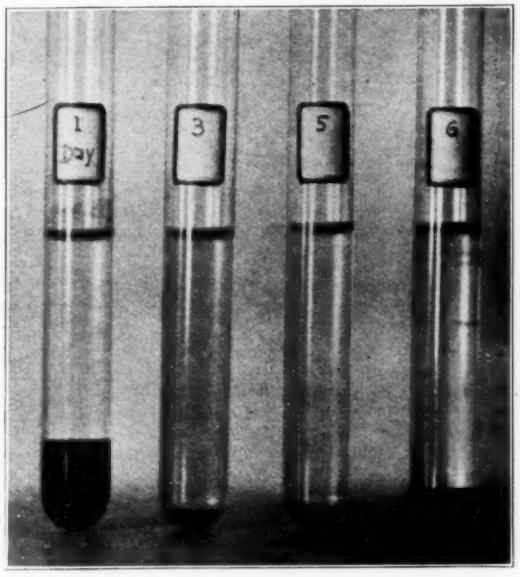

Fig. 3.

amount of wash water a solution of potassium ferrocyanide. While originally a heavy precipitate is obtained as shown in the left hand tube of Fig. 3, it becomes progressively less until, at the end of four or five days, it disappears entirely. This is shown in the tube marked *5* in Fig. 3. If, now, when the loss of iron has dropped to this zero point, some hydrochloric acid is added to the contents of the diffusion thimble, the iron reappears in the surrounding water as shown in the right hand tube of Fig. 3.

The change to the electronegative ferric hydroxide within the diffusion thimble may be followed by dipping strips of filter paper into the diffusion thimble from day to day and noticing the height to which the color ascends. In the first days the iron salt and the water rise together to a great height, but later (after four to ten days) the water still rises, but the now colloid iron comes to rest just over the surface of the liquid as shown in Fig. 2.

III. THE ANALOGY BETWEEN GRAHAM'S EXPERIMENT AND CERTAIN BIOLOGIC FACTS

It is important to point out next the analogies which exist between this simple experiment and certain facts of kidney function. If we will call the contents of the diffusion capsule "kidney parenchyma" and the water surrounding the capsule "urine"—the entirely secondary importance of the diffusion capsule will be pointed out later—the following facts will at once become apparent.

It is obvious, first of all, that there is always derived from the thimble contents a secretion more acid than the medium from which it comes, just as there escapes from the kidney a urine more acid than the tissues themselves. In the later stages of the diffusion experiment an acid secretion is obtained not merely from a medium which is less acid, but from one which is actually "alkaline." The fact that the ferric hydroxide no longer ascends the filter paper means just

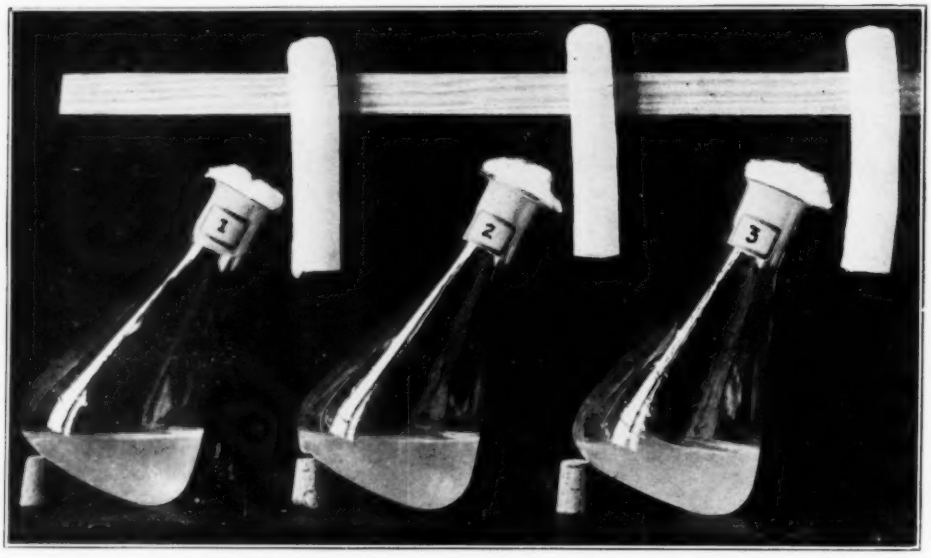

Fig. 4.

this. It is, in other words, an electronegative (alkaline) colloid. But the analogy to kidney physiology and pathology goes further. Ferric hydroxide, except in low concentrations, is a definitely "gelatinous" colloid. Even in the concentrations employed in these experiments it is not an ordinary hydrophobic or "suspension" colloid, but shows distinctly hydrophilic properties. As is now well known, it is this hydrophilic type of colloid which constitutes the bulk of what is termed protoplasm. The "alkaline" ferric hydroxide is, in other words, the analogue of the normal "electronegative" colloids which make up kidney parenchyma.

It is in the later stages of Graham's experiment when an acid secretion of molecularly dispersed substances containing no iron is being derived from a hydrophilic colloidally dispersed matrix that we have the parallel of what happens in kidney and urine under physiologic conditions. The hydrated ferric hydroxide in the presence of traces of salts does not "dissolve" in water (just as no al-

bumin dissolves out of the kidney to appear in normal urine). But if the acid content of the ferric hydroxide is slightly increased, the colloid "dissolves" more easily, shows increased diffusibility and becomes readily miscible with water. In the case of Graham's experiment the iron now diffuses through the parchment capsule and appears in the "urine." This is analogous to what happens in the kidney under pathologic circumstances, as in nephritis. Under the influence of the abnormal production or accumulation of acids (or of similarly acting substances like alkalies, amines, pyridin and urea) in the kidney parenchyma, this not only swells, but shows an increased tendency to diffuse and an increased miscibility with water, and this explains why the material of the colloid matrix, in other words, albumin, begins to appear in the liquid which bathes the parenchyma. The urine, in other words, from which under normal circumstances albumin is absent, now contains such. Accompanying such change, however, there is an increase in the titration or hydrogen-ion acidity of the urine expressive of the increased acid content of the kidney parenchyma. In similar fashion, in Graham's experiment there is a loss of iron during the first hours and while the acid content of thimble mixture and external fluid is high, to become less and less as the more rapid loss of acid allows the iron to change to a hydroxide and to a nondiffusible form. The renewed addition of acid to the colloid iron hydroxide within the thimble again increases the solubility and diffusibility of the iron and at once this manifests itself by a renewed appearance of this metal in the wash water about the diffusion thimble.

The diffusion capsule, it must be clearly kept in mind, is of entirely secondary importance. Even if it were absent, the same results would be obtained, for the parchment thimble only manages in mechanical fashion to keep together the iron solution. Separation through diffusion is the same whether such diffusion is "free" or partially hampered through a colloid membrane.

In the case of a normal kidney the parenchyma itself (the protoplasm of the cells) behaves like the parchment thimble. In nephritis such colloid structures are destroyed and the kidney mixes with the urine just as the noncolloid iron salt would mix with the water, were no parchment thimble present.

IV. VERIFICATION OF THESE PRINCIPLES IN THE CASE OF A SALT-PROTEIN (GELATIN) MIXTURE

In order to show that an entirely analogous behavior is observable if a protein is used instead of an iron salt, the following experiment with gelatin was devised. Gelatin shows, like other proteins, an increasing tendency to liquefy or "dissolve" (an increased degree of dispersion and an increased tendency to diffuse) in the presence of sodium phosphate as the acid or alkaline content of the phosphate mixture is increased from a given low point.[6] The Erlenmeyer flasks of Fig. 4 contained the following gelatin-phosphate-water mixtures:

(1) 50 c.c. 2% gelatin + 3 c.c. 1/1 m NaH_2PO_4 + 4 c.c. 1/1 n H_3PO_4 + 43 c.c. H_2O
(2) 50 c.c. 2% gelatin + 3 c.c. 1/1 m NaH_2PO_4 + 3 c.c. 1/1 n H_3PO_4 + 44 c.c. H_2O
(3) 50 c.c. 2% gelatin + 3 c.c. 1/1 m NaH_2PO_4 + 2 c.c. 1/1 n H_3PO_4 + 45 c.c. H_2O

As shown in our previous observations on this gelatin, these mixtures are just liquid to decidedly liquid at 21° C., a fact readily manifest in the photograph.

These liquid mixtures were poured into ordinary parchment diffusion capsules and dialyzed against distilled water for a number of days. On the fourth day of the dialysis the gelatin in the diffusion thimble of *3* had become semisolid, while that in the remaining thimbles was still liquid. On the fifth day the gelatin from the third mixture was solid, that in the second semisolid, while that in the first was still liquid. At the end of ten days all were solid.*

To understand what has happened it is only necessary to call to mind Richard Maly's[7] classical observations on diffusion. In order to get a physicochemical counterpart of the production of a secretion (like gastric juice or urine) more acid than its source (the glandular parenchyma or the blood) he studied the separation which occurs when free diffusion or diffusion through a parchment membrane is permitted a mixture of acid with an acid salt or one of an acid salt with a neutral salt, etc. Generally speaking, the more acid constituents of such a mixture leave it first, leaving behind the more neutral compounds. It is this principle which is utilized in the experiment described above. From a phosphate mixture containing phosphoric acid, the phosphoric acid diffuses out sooner than the acid phosphate; while from the acid phosphate itself an acid fraction diffuses out leaving behind disodium phosphate. As this occurs, the previously liquid gelatin becomes more and more viscid until, as neutrality is approached, the mixture becomes solid.

The experiment shows in reverse order the state which characterizes the kidney in nephritis and normally. As often emphasized before,[8] albuminuria represents a "solution" of the previously insoluble kidney and is brought about through an accumulation in the kidney parenchyma of materials like acids (alkalies, amines, pyridin or urea). The continuance of the kidney in its normal solid state is dependent upon the maintenance in this structure of a reaction more nearly neutral. In the experiment described above this "normal" state of the kidney is represented by the solid gelatin in the diffusion capsule at the end of the experiment; the nephritic state, by the liquid gelatin readily miscible with water with which the experiment was started. While the urine of the carnivora is for most of each day and normally more acid than the kidney parenchyma or the blood, this acidity is greatly increased under the pathologic circumstances which are associated with "nephritis." These facts, too, may be readily observed in the described experiment.

The more acid nature of the "secretion" in the experiment described above as compared with that of the "parenchyma" (the gelatin mixture) may be observed at any stage of the diffusion by adding some indicator like methyl red or litmus to the capsule contents and to the wash water surrounding the capsule. While I hold that there are serious objections to be raised against the application of indicator methods to definitely colloid systems and to the interpretations which are ordinarily made of such findings[9] it is nevertheless true that the acidity of the wash water about the gelatin always seems decidedly higher than that of the thimble contents. With the progress of the experiment the absolute acidity of the wash water gradually falls. While originally, for example, methyl red is turned

*To reduce liability to infection with liquefying organisms it is well to make up the gelatin mixtures in sterile form, to soak the diffusion capsules in boiling distilled water before use and to keep the diffusion flasks stoppered with sterile cotton plugs.

violently red, this indicator shows only a reddish brown color when diffusion has been allowed to progress for several days.

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A REPORT OF FIVE CASES OF POISONING BY NICOTINE

By William D. McNally, A.B., Chicago, Ill.

DEATH by nicotine poisoning is very infrequent, which is all the more remarkable when we consider the large quantity of tobacco consumed each day. Nicotine is a liquid alkaloid and is the chief poisonous basic principle in tobacco, existing in all parts of the plant, but principally in the leaves which contain from 0.6 to 8.0 per cent, the larger proportion being found in the less esteemed varieties. One cigar contains a quantity of nicotine which would prove fatal to two persons if directly injected into the circulation. Witthaus[1] reports that he was able to find references to only six instances of poisoning by the alkaloid nicotine in the human subject, all of which were fatal. One of these cases, Affaire-Bocarmé, is of special interest as it was here that Stas first used his process for the separation of alkaloids from organic mixtures.

The literature contains many references to tobacco poisoning where tobacco had been swallowed with suicidal intent, accidental poisonings, as in tobacco enemata in the treatment of constipation and pinworms, the use of too much snuff, external applications of leaves to wounds and the use of tobacco powder sprinkled on the skin for favus, Weidanz[2] reporting the death of one child and the serious illness of two others, from this last source. Less severe poisonings have been noted by nearly every one upon beginning the use of tobacco, where the peripheral and nauseant actions predominate. Even chronic smokers often experience ill effects and pain from smoking. Husemann[3] cites a case seen by Hellwig of two brothers who died after continuous smoking of seventeen and eighteen German pipefuls of tobacco. Sonnenschein (quoted by Weidanz[2]) relates the cases of two suicides in which death took place in three and five minutes respectively after swallowing one to two ounces of tobacco. Merriam[4] alludes to an instance of death in a child from the incautious employment of a strong decoction of tobacco, as a lotion for ringworm of the scalp. A man[5] died three

hours after using a tobacco decoction for the cure of an eruptive disease. Grahl[6] of Hamburg relates a case where an ounce of tobacco was boiled in water fifteen minutes and the infusion was administered by the advice of a female quack. The individual was seized in two minutes with vomiting, violent convulsions, and stertorous breathing and died in three-quarters of an hour. After using an infusion of two ounces of tobacco in mistake for two drams, a woman[7] died immediately. M. Travagot[8] records a case where two ounces and one dram were used in place of a dram and a half for an injection in a man affected with ascarides. Death occurred in eighteen minutes. In another case[9] a man died in one hour after an enema of two drams of tobacco in eight ounces of boiling water. A dram is reported as having caused death in one case[10] in a few hours after injection and the same amount to have caused death in thirty-five minutes.[11] Reynolds[12] reports a singular case of nicotine poisoning in which tobacco had been accidentally dropped into food warming on the stove. The food was given twice to a baby five months old, the baby became cyanotic, vomited twice, extremities were cold and clammy to the touch, pulse weak and irregular. Death occurred 13½ hours after the first feeding. In the majority of the cases reported, death was caused by infusions of tobacco. Feil[13] reports a case of a woman, 52 years of age who took an insecticide containing 12 per cent nicotine in mistake for cascara, dying in twenty minutes.

Death occurred in nearly all of the cases of nicotine poisoning within a few minutes to a few hours, although I find record of one instance in which death did not occur for two days after the drinking of wine in which Spanish snuff had been placed by a practical joker, causing the death of the French poet Santeul.[14] In another instance where death did not occur in a few hours, a child,[15] age three, used for an hour an old pipe for blowing soap bubbles. Symptoms of poisoning developed and the child died on the third day.

During the last two years in Cook County, Illinois, five cases of poisoning have occurred from taking by mistake for whiskey, insecticides containing nicotine. The free alkaloid is a colorless oily liquid which rapidly becomes brown in color, closely resembling whiskey in appearance, which accounts for its accidental consumption. The first three poisonings occurred in men employed in green houses who drank the insecticide and as the organs were not analyzed, I will briefly state the circumstances:

CASE 1.—A man drank an unknown quantity of liquid containing 44.84 per cent nicotine. The concentrated solution of the insecticide was kept in a cognac bottle, a small portion of which was diluted in a large volume of water for spraying lettuce and radishes. A similar bottle containing liquor was kept on the shelf alongside the insecticide. The man took one swallow of the liquid, realizing immediately his mistake, ran 75 feet to the house, raised a bottle of milk to his lips, dropping dead before he was able to take a drink of the milk.

CASE 2.—Under the influence of liquor, a man went to the kitchen of his home and drank an unknown quantity of insecticide containing 43.24 per cent nicotine from a whiskey flask, causing death in a few minutes.

CASE 3.—The circumstances are very similar to that of Case No. 2, although the man was not intoxicated. The insecticide was not analyzed, but from the minutes of the coroner's inquest it was learned that the insecticide contained nicotine and was used for spraying plants.

CASES 4 AND 5 occurred on Nov. 7, 1918, at Morton Grove Illinois. Two men, celebrating the first report of the armistice, visited the home of a mutual friend, who was asked to give them a drink of liquor. Three bottles of beer were opened, the host then recalled that he had a bottle of whiskey in the pantry, which he produced and poured out three whiskey glasses full. The capacity of the glasses was 90 c.c. and were said to have been two-thirds filled with the fluid from the whiskey flask. The two visitors drank about half of the portion given to them, one making the remark "what funny tasting booze." Both men had a sensation of choking, dropped to the floor gasping for air, became cyanotic, dying within five minutes. Neither of the men vomited. One of them had a frothy mucus coming from his mouth and nose and had urinated and defecated. The host claimed he drank some of his liquor and immediately produced emesis by drinking a large quantity of salt and water.

A postmortem examination upon the bodies, Cases 4 and 5, made three hours after death showed an intense hyperemia of all of the organs. The stomach was highly congested in both men, having the odor of alcohol. Upon opening the thoracic and abdominal cavities, there was no distinct odor of nicotine. The head was not posted. The stomachs were removed for chemical examination.

The stomach and content of E. N., weighed 345 grams. A volatile distillate of 200 grams of stomach and content showed the presence of 0.4465 grams of nicotine, making a total of 0.7702 grams in the organ.

The stomach and content of F. P. weighed 659 grams. From 115 grams I recovered 0.8673 grams of nicotine, a total of 4.9609 grams.

The whiskey flask from which the nicotine was obtained, contained a light brown colored liquid, of a strong alkaline reaction with the characteristic odor of stale tobacco smoke or odor from an old pipe. A volatile distillate gave 39.84 per cent of nicotine using the silicotungstic method.

The nicotine was identified in the distillates from the stomachs and flask by chemical and physiologic tests. The most delicate test is that of the silicotungstic acid method. At a dilution of 1 in 300,000 of nicotine in the presence of one-tenth of one per cent of hydrochloric acid, an opalescence appears almost immediately. Upon standing, a definite crystalline form will appear (Bertrand and Javillier[16]). Silicotungstic acid does not form such insoluble precipitates with all alkaloids.[17] Conine, for instance, which is of interest in this connection for the reason that it is also volatile with steam, yields no precipitate with silicotungstic acid at dilutions greater than 1 in 5,000.

Gold chloride produces a yellow or brown, amorphous precipitate in neutral solutions of nicotine salts, which is nearly insoluble in acetic or hydrochloric acid, but readily soluble in caustic alkalies. A cloudiness appears slowly at a dilution of 1 in 10,000.

Picric acid throws down an amorphous precipitate of nicotine picrate, which rapidly changes to a mass of crystalline tufts. The picric acid must be added in great excess, if the test is made in small capillary tubes, the nicotine can be detected microscopically in dilutions of 1 in 25,000.

Platinic chloride in strong solutions of the alkaloid or its salts produces a yellowish amorphous precipitate, which on standing becomes crystalline. With more dilute solutions up to 1 in 5000, the precipitate appears more slowly and is crystalline.

Mercuric chloride gives a white precipitate with the free alkaloid, which is at first white and curdy, becoming crystalline upon standing. The precipitate can be obtained in dilutions, 1 in 3000, dissolves in a solution of ammonium chlo-

ride from which it is redeposited. The precipitate is soluble in hydrochloric or acetic acid.

Roussin's reaction is the least delicate of the tests used for nicotine. If an ethereal solution of iodine be added to a solution of nicotine in ether, a brownish precipitate is produced in solutions of 1 in 100, which is converted into long, needle-shaped crystals after standing for some hours. Nicotine reacts energetically with epichlorhydrin when heated to 120, giving a deep red product; in concentrated solutions the reaction is violent. In one-half of a c.c. of a 1 in 500 solution in alcohol is boiled with 2 c.c. epichlorhydrin, a distinct red color is obtained. A lesser quantity may be detected by boiling the mixture for a longer time.

Nicotine produces a cloudiness or precipitates with the general alkaloidal reagents. Tannic acid gives a white precipitate soluble in warm hydrochloric and reappears as the solution cools. Its delicacy, according to Wormley[18] is 1 in 10,000. Phosphomolybdic acid produces a cloudiness up to 1 in 40,000 in acid solution. Wagner's reagent in an acid solution gives a reddish brown precipitate 1 in 250,000 (Wormley). Mayers' reagent in a neutral solution of the alkaloid gives a white amorphous resinous precipitate in dilutions 1 in 15,000 (Dragendorff).

Conine gives negative results with Roussin's reaction, gold and platinic chlorides in dilution over 1 in 100, silicotungstic acid in dilutions greater than 1 in 5000, and Schindelmeiser's reaction. The odor of the distillate with nicotine is so characteristic that it would hardly be confused with the mousy odor of conine.

Physiologic Tests.—If 1 c.c. of 1 in 1000 solution be injected into a 30 gram frog, in about eight minutes a peculiar position will be assumed, the forelegs will be pressed backwards against the sides and the hind legs will be drawn up so that the thighs stand at right angles to the frog's body and the legs will become fixed so that the feet rest upon the back. If the legs are pulled out, they will immediately resume the above position when released. There will be slight muscular spasms in the hind legs. The respiration is first accelerated, then becomes slow. Using this method 1 c.c. of a 1 in 500 solution (made from a 38.94 per cent solution of nicotine) the above described symptoms were obtained. With an injection of ½ c.c. of the original solution from the flask (38.94 per cent nicotine), a severe clonic convulsion was caused, followed by an immobility of the position noted above from the injection of a weaker solution. The respiration was arrested without an acceleration, the heart continuing to beat. The frog is very susceptible to the action of nicotine, and as no elaborate apparatus is required to elicit the above reaction, the unknown drug should be first tried on the frog.

During the examination of the stomach and content of E. N., it was noted that the distillation had to be continued until a thousand or more c.c. of distillate were collected, before obtaining a negative test with silicotungstic acid. When a portion of the distillate is titrated with standard acid, using iodoeosin as an indicator, the results obtained were uniformly higher than by the gravimetric silicotungstic acid method. In this laboratory I have adopted the following method for the determination of nicotine in organs; 100 grams of macerated tissue are

placed in a Kjeldahl distilling flask and a sufficient amount of water and caustic soda is added to make the solution strongly alkaline. A few small pieces of pumice are placed in the distilling flask and the distillation carried on with a rapid current of steam through a condenser and an adapter, into 15 c.c. of dilute hydrochloric acid (1 to 4) in a large flask. When the distillation is well started, apply heat to distillation flask to reduce the volume of the liquid and hasten distillation. The distillation is continued until a few cubic centimeters of the distillate collected from the condenser, after the removal of the adapter, shows no cloud or opalescence when treated with a drop of silicotungstic acid solution, followed by a drop of hydrochloric acid. The distillate is made up to a definite volume and filtered through a large dry filter paper. A portion of the filtrate should be tested with methyl orange, to be certain that the solution is acid. From a standard burette an aliquot portion is withdrawn into a beaker, adding for each 100 c.c. of liquid, 3 c.c. of dilute hydrochloric acid (1 to 4) adding 1 c.c. of a 12 per cent solution of silicotungstic acid for each 0.01 gram of nicotine supposed to be present. Stir thoroughly and let stand for eighteen hours. The crystalline precipitate is filtered onto a quantitative filter paper and washed with cold water containing 1 c.c. of concentrated hydrochloric acid per liter. A portion of the filtrate should be tested with a few drops of nicotine distillate to prove excess of silicotungstic acid. Wash the precipitate several times until no more opalescence appears when a few cubic centimeters of fresh filtrate is tested with a few drops of the nicotine distillate. The wet paper and precipitate is transferred to a weighed platinum crucible, drying carefully and finally burning off the carbon at as low a temperature as possible; the crucible is then ignited over the full heat of a Bunsen burner, followed by the heat of a blast lamp for five minutes. Cool in a desiccator; the weight of the residue multipled by 0.114 gives the weight of nicotine in the aliquot taken for precipitation.

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ECTOPIC ADENOMYOMA OF UTERINE TYPE (A REPORT OF 10 CASES)

By Arthur E. Mahle, M.D., and Wm. Carpenter MacCarty, M.D.,
Rochester, Minn.

IT is evident, regardless of the amount of literature which has been written on the subject, that the importance of adenomyoma has not been recognized either clinically or surgically.

Adenomyomas of the uterus and tubes and those extending into the broad ligament were described before 1894 by Babes, Breus, Diesterweg, and others. In 1896 von Recklinghausen's complete work, "Die Adenomyome und Cyst-adenomyome der Uterus und Tubenwandung" appeared. Adenomyomas of the round ligament, ovarian ligament, rectovaginal septum, and ovary[14] were reported in the latter part of the nineteenth century. So-called adenomyomas occur also in the stomach and intestines, and have been described in the kidney and gall bladder. In the latter cases, the glandular elements resemble those of the tissue in which they are found.

Cullen, in 1908, in his book, *Adenomyoma of the Uterus,* published a review of 83 cases of adenomyoma occurring in 1283 cases of fibromyomas of the uterus; about 5.7 per cent of all the cases. In 1919, MacCarty and Blackman reported a total of 211 cases of adenomyoma in 3398 fibromyomas of the uterus; 6.43 per cent of all the cases.

In 1916, Cullen published his comprehensive work, *The Umbilicus and Its Diseases,* in which he collected all the cases of adenomyoma of the umbilicus reported up to that time, thirteen in number. In his opinion, it was doubtful whether four of these cases should be included in the group.

The 10 cases herewith reported were extrauterine and extratubal tumors, diagnosed at the time of operation as adenomyomas. These growths contained glandular portions resembling typical uterine mucosa, surrounded by a fibrous connective tissue, and smooth muscle stroma, the latter in varying amounts. The distribution of the tumors was as follows: Umbilicus 1, abdominal wall, 2, sigmoid 1, groin 2, and rectovaginal septum 4.

ADENOMYOMA OF THE UMBILICUS

Case 1.—This patient, aged forty-two, had been married for twenty-three years, during which time she had been pregnant four times, the last pregnancy occurring thirteen years before. She had noticed a growth in the "navel" four years before, but it had disappeared until several months before examination. At this time she noticed a hard, bluish tumor, which seemed to be growing larger, "broke open", and discharged a bloody serous fluid. The tumor became quite painful at the time of menstruation.

On examination, the enlargement was found, as described, with bluish areas beneath an intact epidermis, and, clinically, it was considered a "suspicious tumor." Excision of the tumor was followed by a Mayo operation for umbilical hernia. The tumor had no connection with the peritoneum or any abdominal viscus. (Plate I, Fig. 2. Plate II, Figs. 1 and 2.)

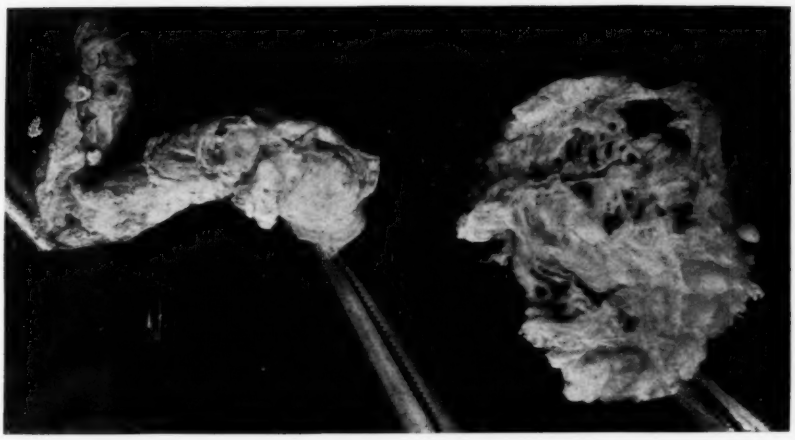

Fig. 1.—(Case 2, 251296.) Adenomyoma of tube and abdominal wall. The thickened portion of tube (adenomyoma, Plate II, Fig. 3) was adherent to adenomyoma of abdominal wall. (Plate II, Fig. 4.)

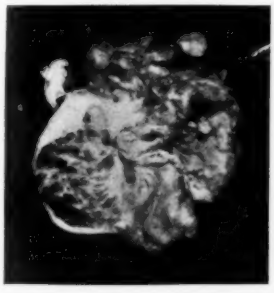

Fig. 2.

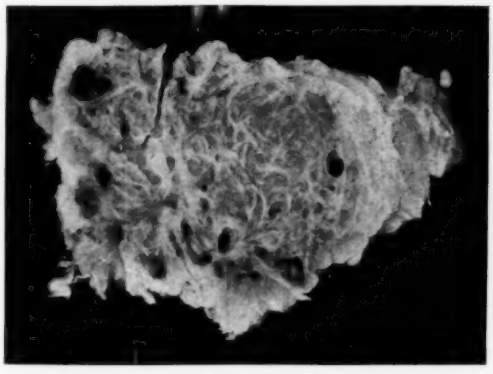

Fig. 3.

Fig. 2.—(Case 1, 261880.) Adenomyoma of the umbilicus cut in cross sections, showing small cystic areas filled with dark brown pigment.

Fig. 3.—(Case 3, 177844.) Adenomyoma of the abdominal wall with white fibrous bands of connective tissue extending through substance of tumor with cystic areas filled with dark brown pigment.

Fig. 4.—(Case 5, 109474.) Adenomyoma of the groin.

PLATE I.

Some patients in the cases of adenomyoma of the umbilicus reported by Cullen gave a history of enlargement of the tumor at the time of menstruation, and one patient noticed a bloody, serous discharge which occurred during catamenia. This author because of the very close resemblance, pathologically, of these tumors, to adenomyoma of the uterus, believes them to have originated from misplaced uterine mucosa or from remnants of Müller's ducts. Goddard, in 1909, expressed the same opinion. While this may be correct, we believe that

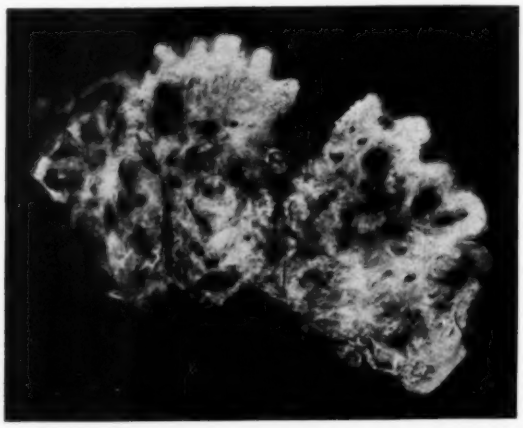

Fig. 5.—(Case 8, 29640.) Adenomyoma of the rectovaginal septum with epithelium intact over tumor mass.

Fig. 6.—(Case 6, 281149.) Adenomyoma of the groin.

Fig. 7.—(Case 10, 277751.) Adenomyoma of the rectovaginal septum with small cystic areas filled with dark brown pigment.

PLATE I.

up to the present time no conclusive evidence has been offered, and that the real origin of these tumors is not positively known. Of the cases which have been reported previously, all have been cured by simple excision of the tumor. Our patient, who was operated on quite recently, has been relieved of all symptoms, but not sufficient time has elapsed to assure a permanent cure.

ADENOMYOMA OF THE ABDOMINAL WALL

The two patients with adenomyoma of the abdominal wall had been operated on elsewhere for retroversion of the uterus, for which one had an internal shortening of the round ligaments, and the other a ventral suspension.

CASE 2.—This patient, aged thirty, complained of a tender lump, of two years' duration, in the lower abdominal wall, under a previous laparotomy scar. The lump was painful at the time of menstruation.

On examination a palpable mass, 3 cm. in diameter, was found beneath the lower end of a median laparotomy scar; this was hard, nodular and painful to touch. It was apparently not attached to the uterus, and, clinically, was thought to be a fibrous tumor in a previous laparotomy wound.

At operation, the mass was removed; it extended through the abdominal muscles, and was attached to the left tube about 4 cm. from the uterine horn. (Plate I, Fig. 1, Plate II, Figs. 3, 4, 5 and 6.)

CASE 3.—This patient, aged forty-six, had had a ventral suspension performed several years before and had been pregnant nine times, the last pregnancy occurring ten years before. She complained of lumps in the abdominal wall, which she had noticed for the last year. These lumps had not grown noticeably larger, but were always painful following menstruation.

On examination, a mass was found in the suprapubic region, apparently in the abdominal wall, movable with it, and possibly connected with the fundus of the uterus. Clinically, it was thought to be a fibrous growth, attached to the abdominal wall on a previously ventro-suspended uterus.

At operation, the fundus of the uterus was found attached to the abdominal wall. The tumor, 8 cm. in diameter, was situated to the right of the midline, and extended down to the right side of the uterus. It was solid, with glandular, cystic areas filled with black pigment. Because of its extension into the retroperitoneal tissue, and apparent inoperability, only a piece of tissue 6 cm. in diameter was excised for diagnosis. (Plate I, Fig. 3. Plate II, Fig. 7.)

In the cases of adenomyoma of the abdominal wall it was not possible to trace a direct continuity of uterine endometrium to the adenomyoma. Cullen, however, has shown that in many adenomyomas of the uterus, this relationship between the endometrium and the adenomyoma could be demonstrated. In Case 2, the adenomyoma of the tube was adherent to the abdominal wall, and from the similarity of the pathologic picture of the two tumors, one is led to believe that the adenomyoma of the abdominal wall arose from that of the tube. In Case 3, the anatomic relationship to the uterus was established, but their pathologic relationship was not microscopically demonstrated. It is therefore impossible to say definitely that the adenomyoma arose from the uterine endometrium.

ADENOMYOMA OF THE SIGMOID

CASE 4.—The adenomyoma of the sigmoid occurred in a patient, aged thirty-one, who had been married eleven years and pregnant once. She had had an appendectomy, salpingectomy, and partial oophorectomy performed elsewhere. At that time she was told that she had a tumor of the lower bowel which would become a cancer. She presented herself at the clinic because of this tumor. X-ray of the colon, and a proctosigmoidoscopic examination proved negative.

At operation a tumor mass was found encircling the sigmoid, involving a segment of the bowel 4 cm. in length. The sigmoid and the bladder were adherent to a mass around the uterus. Twelve centimeters of sigmoid were removed as well as "tarry" cysts of both ovaries. (Plate III, Figs. 3 and 4.)

Since the mass in this case was not removed and its true pathologic condition was not known, only the anatomic relation between the uterus and the sigmoid was established. Leitch, however, reports a similar case of an adenomyoma

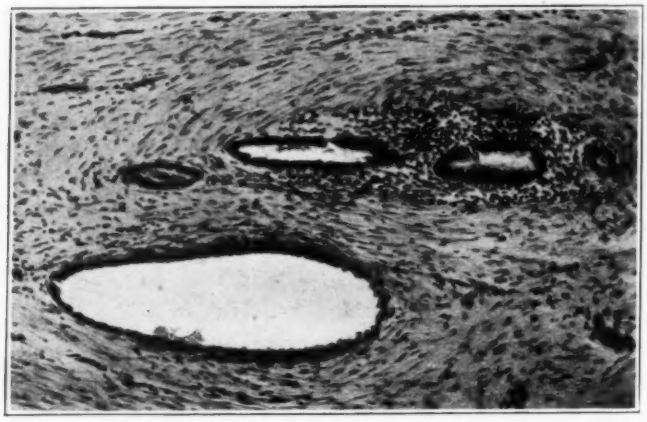

Fig. 1.—(Case 1, 261880.) Adenomyoma of the umbilicus. A group of glands are surrounded by a cellular stroma resembling a typical adenomyoma of the uterus.

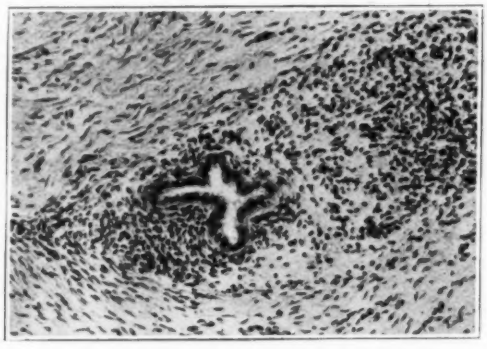

Fig. 2.—(Case 1, 261880.) Same as Fig. 1.

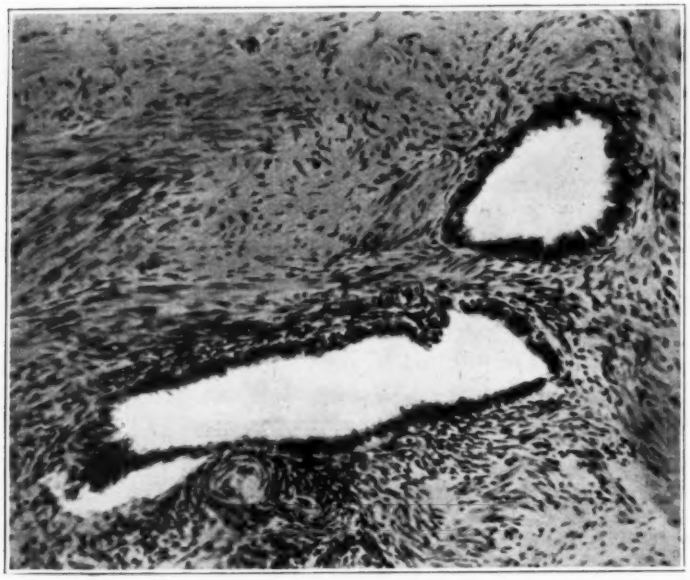

Fig. 3.—(Case 2, 251296.) Adenomyoma of the tube.

PLATE II.

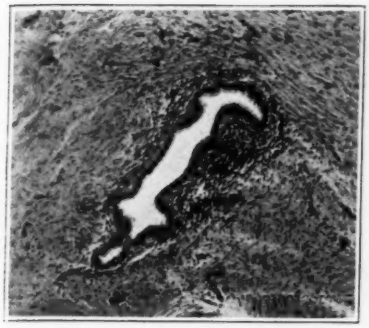

Fig. 4.

Fig. 4.—(Case 2, 251296.) Adenomyoma of the abdominal wall, showing gland lying in a cellular stroma.

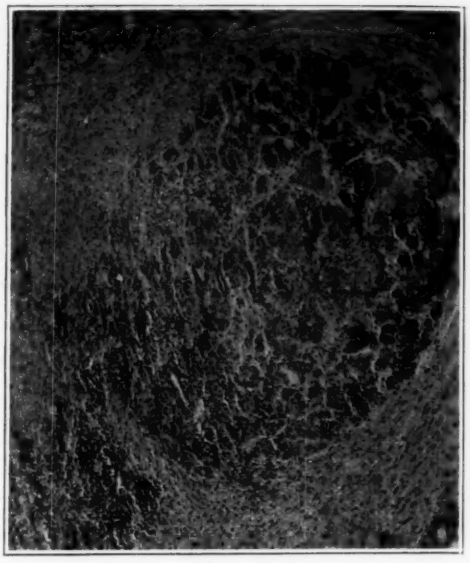

Fig. 5.

Fig. 5.—(Case 2, 251296.) Adenomyoma of the abdominal wall, showing areas of old hemorrhage around striated muscle fibers.

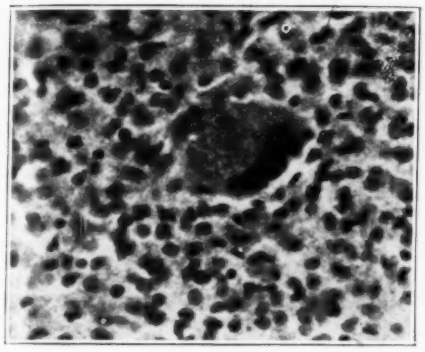

Fig. 6.

Fig. 6.—(Case 2, 251296.) A foreign body giant cell absorbing old hemorrhage, surrounded by endothelial cells containing old blood pigment.

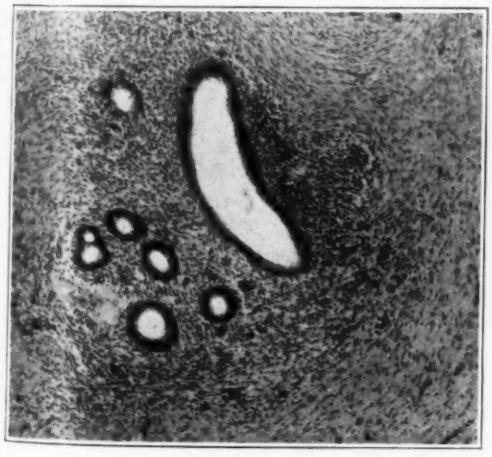

Fig. 7.

Fig. 7.—(Case 3, 177844.) Adenomyoma of abdominal wall.

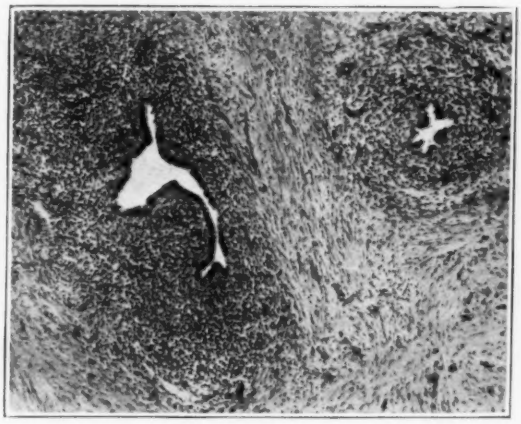

Fig. 8.

Fig. 8.—(Case 5, 109474.) Adenomyoma of the groin. Note typical uterine gland surrounded by cellular stroma as seen in uterine endometrium.

PLATE II.

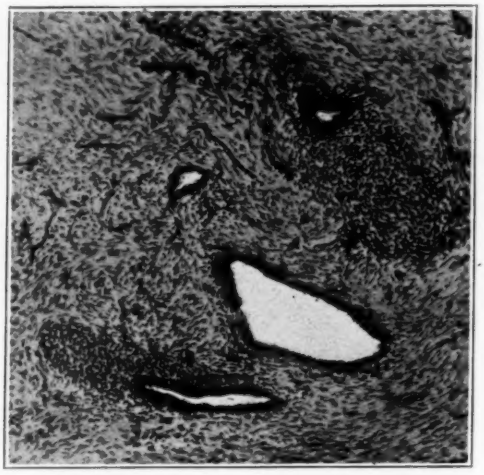

Fig. 1.—(Case 6, 281149.) Adenomyoma of the groin.

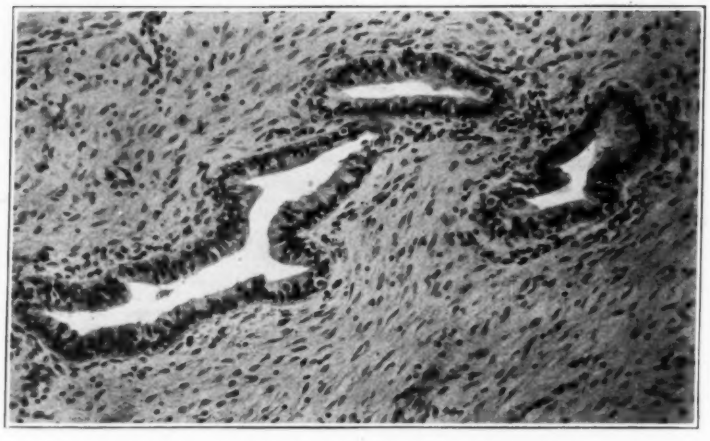

Fig. 2.—(Case 7, 144034.) Adenomyoma of the rectovaginal septum, showing glands surrounded by smooth muscle.

Fig. 3.—(Case 4, 250372.) Adenomyoma of the sigmoid.

PLATE III.

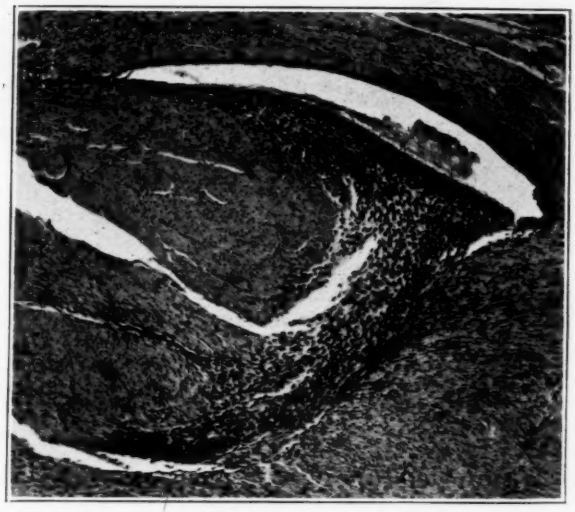

Fig. 4.—(Case 4, 250372.) Adenomyoma of the sigmoid, showing cellular stroma invading muscle fibers. Note old blood and serum in lumen of the gland.

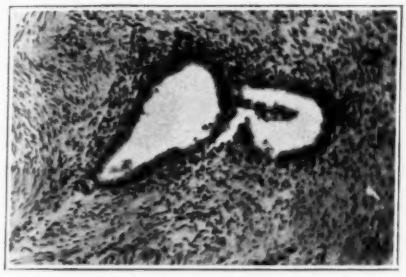

Fig. 5.—(Case 10, 277751.) Adenomyoma of the rectovaginal septum.

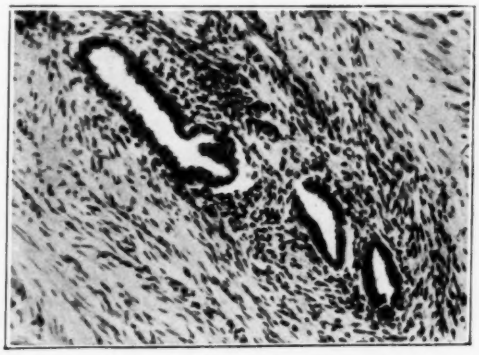

Fig. 6.—(Case 9, 101953.) Adenomyoma of the rectovaginal septum.

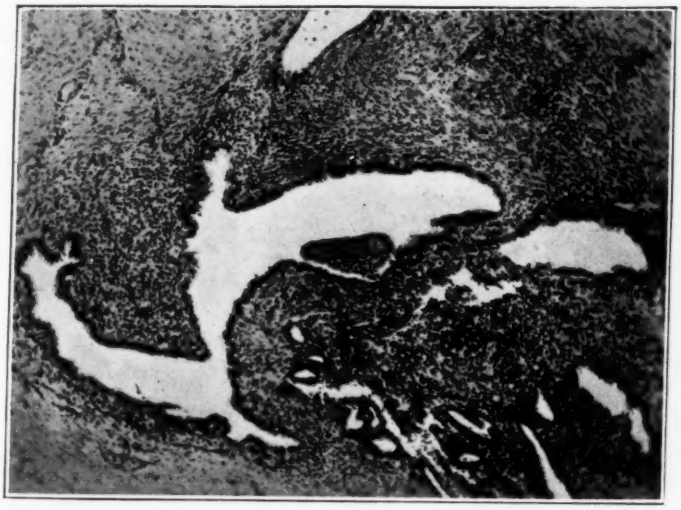

Fig. 7.—(Case 8, 29640.) Adenomyoma of the rectovaginal septum, showing a small cavity surrounded by a cellular stroma containing small glands.

PLATE III.

of the sigmoid, in which this viscus was attached to the posterior wall of the uterus. Specimens from the uterus and the sigmoid showed the same pathologic picture. The simple facts in these 2 cases lead us to believe that the adenomyomatous tissue invaded the sigmoid from the adenomyoma of the uterus. In neither case was the mucosa of the sigmoid involved, thus indicating that these growths infiltrate from the outer bowel wall and grow between muscle fibers and into the loose connective tissue of the serosa and submucosa.

ADENOMYOMA OF THE GROIN

Cases 5 and 6 were classified as adenomyoma of the groin, although in Case 6, the tumor was located in the lower right abdomen beneath a scar due to an appendectomy.

CASE 5.—This patient who had been married eight years, but had never been pregnant, had come to the clinic five years before because of sterility. At that time her examination was negative, except for several small nodules which were felt behind the uterus. She returned four years later, complaining of a large gland in the right inguinal space which she had noticed only a short time, and which at the time of menstruation became larger and tender.

On examination, a tumor mass was found, fairly hard and tender to touch, presumably a gland, 4 cm. in diameter, in the right inguinal group of lymphatic glands.

This patient returned 1 year later. The gland had enlarged, and she complained of some pain in the back and lower abdomen. Since nothing further was found on examination than had been noted on former visits, it was decided to excise completely the tumor in the groin. The tumor was diagnosed pathologically as adenomyoma. (Plate I, Fig. 4. Plate II, Fig. 8.)

CASE 6.—This patient, aged fifty, single, had had an appendiceal abscess drained twenty-five years before. She complained of a palpable tumor, a right inguinal hernia, and a thickening of the appendiceal scar. Four years before she had noticed in this scar two small lumps, slowly increasing in size, which became painful at the time of menstruation.

On examination, some induration of the appendectomy scar was noticed, as well as a right inguinal hernia and a large pelvic tumor, presumably a fibromyomatous uterus.

At operation, the fibromyomatous uterus was removed and the inguinal hernia repaired from within. Later, under local anesthesia, the appendectomy scar was dissected out. The specimen appeared as an indurated mass resembling a keloid, but underneath were cystic areas filled with brown fluid. This tissue, which extended down to the femoral ring, was diagnosed pathologically as adenomyoma. (Plate I, Fig. 6.)

Those adenomyomas of the groin, which have been reported in the literature, were, in most instances, connected with the round ligament.[4] The tumors in our cases showed no relation to this structure, but were situated lateral to it and, at operation, no association could be established, either to the round ligament or any structure closely related to the uterus.

ADENOMYOMA OF THE RECTOVAGINAL SEPTUM

Cases 7, 8, 9, and 10 of adenomyomas of the rectovaginal septum comprise the entire number observed in the Mayo Clinic during a period of ten years. Since so much has been written concerning such tumors, we shall not describe our cases in detail, but consider them in a group. The importance of the condition should, however, be emphasized.

CASES 6 TO 10.—The average age of these patients was thirty-seven, ranging from thirty-three to forty-five. Two patients were married, one of them was sterile, the other

had been pregnant three times. Menstruation in each was regular with no intermenstrual bleeding. Two had had previous gynecologic operations elsewhere, one a myomectomy and a ventral suspension, and one a vaginal hysterectomy for hemorrhage and a cervix suspicious of malignancy.

One patient only came for examination because of symptoms traceable to the tumor, which were pain in the rectum at the time of menstruation and difficulty in defecation. In all the other cases the adenomyomas of the rectovaginal septum were found in the course of routine physical examination; all showed a tumor situated in the rectovaginal septum, varying in size from 0.5 to 3 cm. in diameter. No case showed an involvement of the rectal or vaginal mucosa. Only one had a polypoid tumor formation raising the vaginal mucous membrane on the posterior vaginal wall. The diagnosis of adenomyoma was not made clinically in any case.

The postoperative results are of interest, in that the patient with definite symptoms from the tumor, complained, three years later, of a pain in the vagina associated with a greenish-yellow discharge. This patient had been treated with radium with questionable results. The other patients have no subsequent history of note. (Plate I, Figs. 5 and 7. Plate III, Figs. 2, 5, 6, and 7.)

Pathologically, extrauterine adenomyomas are identical in appearance regardless of where they are found. They differ grossly from adenomyoma of the uterus, in that the cystic areas are larger and the contents darker brown. (Plate I, Figs. 1 to 7.) Grossly, the tumors are solid, fibrous, and of a light gray color. Here and there, white bands extend into the tumor substance, while between these bands are areas, dark brown to almost black, varying in size from the head of a pin to cystic areas 1 cm. or more in diameter. On pressure, a dark brown fluid exudes from the larger cystic areas.

Microscopically, the stroma consists of fibrous connective tissue and smooth muscle fibers, the latter in varying amounts. Within the stroma are gland spaces lined with cylindrical epithelium. Some glands are surrounded by a very cellular stroma, the cells of which are regular with round or oval nuclei resting in a very fine reticulum, while other glands are immediately surrounded by smooth muscle or connective tissue. In some portions of the tumor substance there is marked evidence of recent and old hemorrhage. In the latter areas are clumps of endothelial cells filled with old blood pigment and in one of these areas a typical foreign body giant cell is seen enclosing a mass of blood pigment. The adenomatous portions of these tumors with their cellular stroma are identical with uterine endometrium. (Plates II and III.)

Clinically, these tumors give no consistent group of symptoms on which an accurate diagnosis can be made. However, their location and their slow growth, extending over a period of years, suggest benign tumors. Further, the occasional relation to the time of menstruation, of pain or swelling of the tumor, or less frequently a bloody discharge should be very suggestive of adenomyoma.

Surgically, adenomyomas, regardless of their remarkable infiltrative characteristics, should be differentiated from malignancy. Especially is this true of tumors in the pelvis, adherent to the sigmoid or the abdominal wall or other structures. Adenomyomas may be recognized grossly in most cases by the fibrous stroma which contains cystic areas filled with a bloody, dark brown, or serous fluid.

The pathologist should distinguish adenomyoma from carcinoma by the regularity of gland structure with normal differentiated epithelial cells without

mitoses, and, in most tumors, by the characteristic stroma surrounding the glands. He should also recognize that they are benign tumors, that they grow by invasion, and do not metastasize.

It is also of interest that in our series of cases the true nature of the growth was not suspected before operation. Two were diagnosed malignant, one questionably malignant, and the others were left to be diagnosed pathologically at the time of operation.

All cases occurred in patients between the ages of twenty-nine and fifty. Pregnancy apparently had no influence, for six of the cases occurred in nulliparous women.

Of the 10 patients, 6 gave a history of symptoms directly referable to the tumor. One stated that a tumor mass was found during operation, elsewhere, for other symptoms of which the patient complained at that time. The remaining 3 patients had adenomyomas of the rectovaginal septum which were so small that they were giving no trouble. In these cases, the symptoms, when they were noted in the history, were enlargement, pain in the rectum or vagina at the time of menstruation, or a vaginal discharge.

Our knowledge of the origin of these tumors at present is only theoretical. von Recklinghausen thought they arose from the Wolffian body or duct. Ivanoff, and later Aschoff, according to Lockyer, suggested their origin from the epithelium of the peritoneum of the different regions in which these tumors are found. Cullen believes them to be from remnants of Müller's ducts or misplaced uterine endometrium. No doubt more extensive work on the embryology of the genitourinary tract will solve this interesting problem.

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THE IMPORTANCE OF BIOLOGIC CLASSIFICATIONS IN EPIDEMIOLOGY

By L. C. Havens, M.D., Iowa City, Iowa

TO the epidermiologist the prime factor of importance in the study of pathogenic bacteria is their mode of transmission from one person to another. Epidemiology is concerned primarily with the study of the sources of infection and means of transmission of the diseases due to these organisms with the purpose of increasing knowledge of methods for their prevention. The following considerations are set forth to show the practical necessity, in epidemiologic work, of a biologic classification of the microorganisms which cause disease.

A morphologic classification into Coccaceæ Bacteriaceæ and Spirillaceæ is, of course, fundamental. A morphologic study furnishes a practical basis for nomenclature and comparison. However, such a grouping is no more than fundamental. We know that there are a goodly number of species of streptococci or of the bacilli of the colon-typhoid group, for example, that can not be differerentiated morphologically but that can be shown to be unlike by their cultural characteristics, that is, they produce different appearances in their growth on artificial media, their ability to split sugars and the higher alcohols varies, and thus we have a further means of identification.

These two methods, the study of cultural and morphologic characteristics, together with tinctorial properties, have, until recently, been our only means for the recognition of bacteria, and much progress has been accomplished by their use. With a few striking exceptions, all the bacterial causes of disease have been determined by these methods and in a number of instances, a potent therapeutic agent has been obtained.

In spite of this progress, there is still much that is unknown and this is especially true from an epidemiologic standpoint. Former methods for the classification of bacteria have disregarded the reactions which they arouse in their natural host, the animal body. The study of the measures used by the body to resist infection has progressed rapidly from empirical observations to the status of a separate science. From the observations that many diseases leave the individual resistant to a second attack has developed the science of immunology. One of the well-known basic principles of immunology is that immune reactions are as a rule, highly specific, that is, each race or "strain" of bacteria stimulates the production of antibodies when injected into the body, either naturally or artificially, and furthermore these antibodies are specific for a small group; other groups, although morphologically and culturally identical, being often not included in the antibody formation.

It is rational, then, to expect that a biologic classification would bring out many points of interest in relation to epidemiologic studies of disease. That this may be so is shown by the increased knowledge of the epidemiology of pneumonia following the discovery that the pneumococcus, instead of being one

species, was composed of several distinct biologic types, which, nevertheless, are identical culturally.

Pneumonia was formerly considered an endemic disease, the causative organism being supposedly universally carried in the secretions of the nose and throat and that consequently it was useless to attempt any preventive measures. The main hope of decreasing the incidence of pneumonia lay in increasing individual resistance to the disease by hygienic measures, while attempts to increase resistance by prophylactic vaccination met with failure. It also seemed that pneumonia was an exception to the infectious diseases in that it failed to confer an immunity of any duration, since second and third attacks were common.

Since the discovery of the biologic types of the pneumococcus[1] it has become evident that pneumonia is a true epidemic disease, that the organisms carried universally in normal mouths are commonly some of the less virulent strains of Type IV and that if a person is found to harbor one of the more virulent types it is usually because he has been in contact with a case of pneumonia of that type. That this is not always the case has been shown by a survey at an army camp[2] and a routine examination of surgical cases before operation[3] where carriers of the more virulent types were found. We know that an epidemic of pneumonia due to one type may occur coincidently with an epidemic of a second type. In other words, the epidemiologist is aided in tracing the sources of infection. We know, furthermore, than an attack of pneumonia confers an appreciable immunity of considerable duration and that further attacks are due to other types of the pneumococcus against which no immunity has been produced. Finally experiences in the army[4] in the last year have shown that prophylactic inoculation against pneumonia may be efficient by the inclusion of representatives of all groups in the vaccine.

Another group of organisms which was supposed to be a unit from morphologic and cultural evidence is the meningococci. Formerly the serum treatment of meningitis was followed by many failures, due to the fact that no biologic classification had been worked out, and consequently the serum was often of low titer for the particular strain causing the infection. With a biologic classification representative strains can be used and a more universally potent serum is produced.[5] The British Army, early in the war, had such an experience; the serum which was being used contained no antibodies against the particular strain which caused the epidemic. An investigation of the strains causing the epidemic and the inclusion of representatives in the production of the serum gave successful results. Besides saving a useful therapeutic agent from the scrap heap, the successful classification has been an aid in the search for carriers. The organism in the nasopharynx of the healthy carrier must correspond biologically with the organism causing the epidemic in order to prove the carrier to be a factor in the spread of the infection. A parameningococcus carrier can not be a source of an epidemic due to a "normal" meningococcus.

The epidemiology of streptococcus infections—pneumonia, sore throat, tonsillitis and bronchitis due to this group of organisms—is in much the same state as that of the pneumococcus previous to its classification into groups. It is known that many individuals carry these organisms in their throats and that

chances for infection are constantly present. These organisms belong to several groups culturally, but there seems to be relatively little difference in the degree of infectivity between the various groups. Recently the hemolytic streptococci have been classified biologically[6,7] and it has been found that the cultural characteristics have no relation to the biologic grouping, the serologic types overlapping the cultural groups.[7] A further point which has been brought out in this study which, if corroborated, will prove to be an important epidemiologic factor, is that in a small series of fatal cases of streptococcus pneumonia studied the organisms isolated were all of one biologic type, indicating that this group is much more virulent than the others.

One more illustration will serve to show the practical importance of biologic groupings. Tetanus antitoxin was one of the first therapeutic serums to be used and has long been thought to be highly specific. Yet in a study of tetanus infections in the British Army several biologic groups of the bacillus were found.[8] The standard serum of the United States Public Health Service and of the British Government included only one of these groups, thus explaining the failure of antitoxin in a small number of cases due to the less common types. Tetanus antitoxin, by the inclusion of these other groups, can now be made more completely successful.

That a biologic classification of the colon-typhoid group may be of value to the epidemiologist seems probable. Satisfactory cultural standards for the differentiation of members of this important group are unsatisfactory, especially for differentiation of B. coli of fecal and nonfecal origin. Much clearer light could doubtless be thrown on this group of organisms by a classification according to their biologic characteristics.

One thousand deaths from diphtheria occur every year in New York City alone[9] in spite of the increasing use of antitoxin. An investigation of the serologic properties of the diphtheria bacillus might show biologic differences as in the case of the pneumococcus and the tetanus bacillus.

Only a few striking instances of the value of studying the biologic characteristics of bacteria have been cited. However, the importance of further investigation of the serologic properties of the different pathogenic groups has been shown by these actual cases and the fact that practical interest in these organisms lies largely in this field adds to the interest of such a classification. To the epidemiologist such work as has been done has proved of immense value and every new discovery that aids in the precise recognition of etiologic factors is of the utmost importance.

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LABORATORY METHODS

THE ICE-BOX FIXATION METHOD IN THE PERFORMANCE OF THE WASSERMANN REACTION*

By R. G. Owen, M.D., and F. A. Martin, M.D., Detroit, Mich.

HISTORICAL

WHILE certain radical changes in the technic of the Wassermann reaction have been advocated by Noguchi, Hecht-Weinberg, Stern, and others. the fundamental principles, the original method of Wassermann, Neisser and Bruck is still followed by the majority of serologists.

The most important changes in technic have been the adoption of smaller amounts of the various ingredients which enter into the test, the substitution of plain alcoholic or cholesterinized heart, human, beef and guinea pig, for the original watery liver extract of Wassermann and various refinements in the standardization of the hemolytic system, such as, using a fixed amount of amboceptor and titrating the complement for each set of tests; yet, to date, it has been impossible to get serologists to adopt any uniform method, each man being satisfied with his own peculiar technic and, perhaps, with justice.

Nevertheless, partly because of such varying technic, the Wassermann reaction has been unjustly criticized on account of discordant reports rendered on the same serum by different workers. The true worth of this reaction has been most aptly expressed by Vedder, who says: "One is impressed by the fact that the Wassermann reaction must be a test of most surprising merit to have survived all the clumsy technic that has been perpetrated in its name."

While the use of cholesterinized antigens gives a much higher percentage of positive results in cases of known syphilis, nevertheless the consensus of recent opinion is that such antigens undoubtedly give a not inconsiderable number of strongly positive reactions in cases where syphilis can be definitely excluded. The published figures of such false positive results run as high as 10 to 20 per cent, and the gain in detecting known cases of syphilis is certainly not compensated for by this considerable error. It is far better to get negative results in a few cases of syphilis than it is to attach the stigma of syphilis to an innocent person, for while we serologists insist that the Wassermann reaction should only be considered as one of the most constant symptoms of syphilis, nevertheless we know full well that in most cases the diagnosis is based entirely on results of the blood examination, even weak positive results being considered diagnostic of lues.

Any method, therefore, which will increase the percentage of true posi-

*From the Serological Department of the Detroit Clinical Laboratory, Detroit, Mich.

tive reactions without giving false results in nonsyphilitic conditions should have our most careful consideration.

Such a method, or rather, a variation of the older methods is now coming into use which fulfills these two requirements.

In the original Wassermann method the serum, antigen, and complement were left at 37.5° C. for one hour to permit the fixation of complement. Bordet, Gengou, and Wassermann tried various time limits and also the effect of various temperatures on complement fixation, and found that the process was a gradual one, occurring best at body temperature and they also determined that fixation of complement was practically complete in one hour, while longer periods at this temperature led to deterioration of the complement.

In 1910 Jacobstahl[1] advocated carrying out this first phase of the reaction in the ice box at about 4° C., where the tubes were left for one and one-half hours.

He found 2 per cent more positives, testing 200 bloods, when ice box fixation was used, but apparently missed the main reason for the superiority of such low temperature fixation, namely, that the reagents could be left in contact with each other for much longer periods without deterioration of complement. He did not try periods longer than one and one-half hours, and considered that the increased sensitiveness of his technic was due to better precipitation of the lipoidal extract with absorption of complement, such precipitation occurring better at 4° C. than at 37° C. He gives no details as regards the antigen used.

Guggenheimer[2] ran 623 sera with preliminary incubation at 0° C. and at 37.5° C., keeping both sets at the desired temperature for one and one-quarter hours.

Five hundred thirty four sera showed no difference in the two methods, being either negative or strongly positive; 89 cases showed a difference, but in 69 the difference was mainly a slight quantitative one which in no way affected the diagnosis; 20 cases, however, showed a radical difference in results, 12 being positive only at 0° C. and 8 only at 37° C., these 20 cases being clearly luetic. He used syphilitic fetal liver extract as antigen.

McNeil[3] was the first American observer to report cases tested in this way, and his method was adopted as the standard technic of the New York City Board of Health.

He used crude alcoholic tissue extracts, employing the Wassermann technic with inactivated serum, and found that a fixation period of four hours at 8° C. to 12° C. was best. Testing 466 cases he got 37.7 per cent of positive reactions with incubator fixation against 48 per cent of like results when fixation was carried out in the ice box. He failed, however, to give any histories of these cases, so we are left at a loss as just how to interpret his findings.

Altman[4] also claims to have obtained more satisfactory results when ice box fixation was used. Like Guggenheimer, he reports that certain bloods are positive at 37.5° C. which failed to react at the ice box temperature.

Favorable results with the ice box fixation have also been reported by Leredde and Rubinstein[5].

Coca and L'Esperence[6] report 828 sera run at 37.5° C. for one hour and in the ice box at 7° C. for eighteen to twenty-four hours. They use a special technic of their own devising, with an acetone-insoluble antigen. They report 584 bloods negative with both methods; 203 positive at 7° C. and 37.5° C., while 28 were positive only at the ice box temperature and 13 positive only at 37.5° C.

Smith and MacNeal[7] have reported similar favorable results with the ice box fixation method. They compared the results of cholesterinized antigen at 37.5° C. in incubator for one hour with plain alcoholic heart extract left a similar time at 37.5° C. and at ice box temperature, 8° C., some for four hours, others for sixteen to eighteen hours. They make no remarks concerning the best length of time for the ice box fixation. Testing 110 cases of known syphilis, they found 58 per cent of positives with cholesterinized antigen with incubator fixation against 77 per cent with plain antigen with ice box fixation, and only 32.2 per cent of positives when the plain antigen was used in the incubator method. With 43 patients, probably syphilitic, they got 65.9 per cent positive with cholesterinized antigen, and 75 and 36.3 per cent, respectively, with plain antigen in ice box and incubator. Fifty-nine patients, probably not syphilitic, yielded 40 per cent, 5 per cent, and 1.6 per cent, respectively, by the three methods. Two hundred sixty five nonsyphilitic patients were negative by all three methods.

They used human, beef, and guinea pig hearts as antigen, extracting 10 gm. of tissue with 100 c.c. of absolute ethyl alcohol and diluting this extract with from 10 to 31 volumes of salt solution. No details as to how the antigens were titrated are given.

The high percentage of positive results with cholesterinized antigen in the group "probably not syphilitic" is hard to reconcile with the lower figures given by this antigen in the "known syphilitic" and "probably syphilitic" groups, especially when it is considered that they report no positive reactions whatever with cholesterinized antigens among 265 nonluetic patients. We can not reconcile their 40 per cent of positive results with cholesterinized antigen among probable luetics with their negative results on 265 negative cases.

Their figures would indicate that cholesterinized antigens give no false positives with nonluetics, while yielding a very high percentage of positive results in doubtful cases and lower figures than plain antigen, ice box fixation, with known syphilitics.

It seems to us on theoretical grounds, and this belief is substantiated by our own figures, that cholesterinized antigens will uniformly give a higher percentage of positives in known syphilitics, doubtful, and nonsyphilitic cases.

In a personal communication, Dr. Smith informed us that cholesterinized antigens when used with ice box fixation gave a very high percentage of false positives, and our own investigations fully corroborated his results. These authors do not report any cases strongly positive with incubator method, with plain antigen, and negative with ice box fixation, but they did find 3 specimens giving ++ with plain antigen in ice box which gave ++++ reactions in the incubator test.

Ottenberg[8] considers ice box fixation with plain antigen as giving by far the most delicate and accurate results. He quotes Swift who, in conjunction with

Walker, first advocated cholesterin reinforced antigens in America, as saying that plain antigens with ice box fixation give results identical with cholesterinized antigens with incubator fixation.

With weakly positive sera Ottenberg[8] found that fixation was not as good at 0° C. to 2° C. in ice box as in the incubator but at 8° C. to 10° C. he found results just as good when left in ice box for one hour and even better when a period of four hours was used.

He states that fixation periods of twelve to eighteen hours are not as satisfactory as a four-hour time.

Like Smith and MacNeal and ourselves he finds that cholesterin antigens can not be used with ice box fixation.

Kaliski (quoted by Ottenberg[8]) combines incubator and ice box fixation, leaving the serum, complement, and antigen in the ice box for two hours, followed by one hour in the incubator.

TECHNIC

With our technic we use the following quantities:

1. Serum, 0.1 c.c. freshly inactivated at 56° C. for one-half hour.
2. Complement, (1 to 10 dilution) 2 units.
3. Sheep cells, 5 per cent suspension, 0.5 c.c.
4. Antigen—
   a. Cholesterinized, (1 to 10 dilution) 0.1 c.c. to 0.15 c.c.
   b. Plain alcoholic, (1 to 10 dilution) 0.3 c.c. to 0.7 c.c.
5. Amboceptor, 2 units.
6. Salt solution, q.s. to make vol. of 2.5 c.c.

The amboceptor unit is determined by using 0.5 c.c. of 1 to 10 dilution of complement with 0.5 c.c. of 5 per cent sheep cells and salt solution to make volume up to 2.5 c.c. This amboceptor unit is found after using several pooled complements. Using two units of amboceptor, a daily titration of complement is done and the unit determined. In the actual tests two units of complement and two units of amboceptor are employed.

We have found that such complement titration gives much more reliable results than when a fixed amount of complement is taken as two units and the amboceptor unit determined. If complement be not in excess, the presence of more than two amboceptor units, whether added or present in the serum makes little difference in the results. If, however, a fixed amount of complement be taken as two units and then two amboceptor units are used, the presence of natural hemolysins may cause false negative results, owing to the excess of complement often present in such arbitrarily chosen amounts.

In getting the daily complement dose we use three amounts of amboceptor with increasing amounts of 1 to 10 complement dilution. Thus if two amboceptor units be considered as 0.5 c.c. of a 1 to 1000 dilution, in the daily titration we would test 0.4 c.c., 0.5 c.c., and 0.6 c.c. against increasing doses of complement. The complement unit is then determined from that one of the three titrations which will make the daily dose (2 units) 0.5 c.c. or less, and is used with the corresponding amboceptor dose (2 units). This three-way standardization al-

lows for any variation in the complement in use on that certain day and renders restandardization unnecessary.

We always inactivate the sera within an hour or two of testing them, as we have occasionally had a blood negative with cholesterinized antigen when tested immmediately after inactivation which would react positively if tested twelve to eighteen hours after inactivation.

The antigens are made up daily from the alcoholic extract, blowing the extract into the proper amount of salt solution, a 1 to 10 dilution in salt solution being used.

We determine the antigen value of each antigen by testing it against a series of pooled, strongly positive sera, and find that nearly all cholesterinized antigens, whether made from human, beef or guinea pig hearts, give complete fixation with our method (0.1 c.c. of serum) in amounts varying from 0.01 c.c. to 0.015 c.c. of 1 to 10 dilutions.

Even relatively poor alcoholic extracts were made good by adding cholesterin which seems to act as a sort of stabilizer. To the original alcoholic extracts was added 0.4 per cent of cholesterin.

With the plain alcoholic extracts, the fixation point varies from 0.03 c.c. to 0.07 c.c. Only those giving fixation with less than 0.07 c.c. were considered fit for use.

Having thus found the antigenic unit, we use 10 such units in our tests, provided this amount is not over one-half the beginning anticomplementary dose when tested against pooled sera. In the present work we used 0.1 c.c. of a 1 to 10 dilution of cholesterinized antigen and 0.5 c.c. of the plain.

Our standardization of antigens and our daily titrations are all carried out at 37.5° C., using one hour time periods for fixation and for the second phase of the reaction.

When ice box fixation was employed, the tubes were left at 7° C. to 10° C. for four hours. Amboceptor and sheep cells were then added, and the tubes put in a water-bath at 37.5° C. for twenty to thirty minutes, at which time the negative controls would be cleared. The sets run in the incubator were allowed one hour for fixation, and were read after about one hour, or when the negative controls showed complete hemolysis.

Each specimen was run with three plain alcoholic heart antigens in the ice box method, and in addition, plain and cholesterinized antigen sets were run at 37.5° C. The reactions were graded from 0 to ++++ according to the degree of fixation.

We found that every specimen positive in the incubator set with plain antigen, was equally as strong and often more strongly positive by the ice box fixation method.

We, therefore, do not see the necessity of running duplicate sets in incubator and ice box. We find that from four to six hours in the ice box is amply sufficient for complement binding, and that when bloods are left at this temperature for twelve to eighteen hours, occasional doubtful or weakly positive results are found with known negative bloods.

Intervals longer than four to six hours did not yield any higher percentage of positive results in known syphilis.

RESULTS

We examined sera from 1113 patients. Five hundred of these gave a definite history of lues or else showed symptoms of a syphilitic nature. Among these cases were many giving histories of very long standing, some also had gone years without symptoms, while others had been recently subjected to vigorous treatment. If we had included only the cases of active lues, we feel certain that our percentage of positive results would have been much higher.

Another 500 sera were from patients without any history or symptoms of lues, including cases of tuberculosis, cancer, nephritis, pernicious anemia and diabetes, with and without diacetic acid.

The remaining 113 cases were classed as doubtful, either because of an indefinite history or owing to the fact that the symptoms did not accord with the history or a clinical diagnosis of lues.

We have grouped together as positive reaction those sera giving +++ and ++++ results. The ++ and + reactions have been classed as doubtful.

The results obtained with the 500 cases of known syphilis are shown in Table I.

TABLE I

REACTIONS OBTAINED ON SERA OF 500 CASES OF KNOWN SYPHILIS

TEMP. OF FIRST STAGE	TIME	ANTIGEN	POSITIVE		DOUBTFUL		NEGATIVE	
			No.	%	No.	%	No.	%
37.5° C.	1 hr.	Cholesterinized	383	76.6	23	4.6	94	18.8
37.5° C.	1 hr.	Plain	258	51.6	35	7	207	41.4
7-10° C.	4 hrs.	Plain	353	70.6	16	3.2	131	26.2

It will be noted that while the plain antigen with ice box fixation gives but 6 per cent less of diagnostic reactions than does the cholesterin reinforced preparation, this same plain antigen when incubated at 37.5° C. for one hour, gives only 51.6 per cent of positive results, about 20 per cent less than the figures obtained by the ice box method.

Another point in favor of this technic is the sharpness of the reading found, doubtful reactions not being obtained nearly as often as is the case with the older method.

While the cholesterinized antigen shows 6 per cent more of strongly positive results among this group, we feel that an examination of the cases described below (Table II) will show that from the diagnostic standpoint, the ice box fixation method falls but little below the cholesterin antigen results.

It will be observed that most of the cases positive only with cholesterin were old latent cases absolutely free from clinical symptoms.

The advisability of subjecting these long-standing latent cases to treatment simply on the ground of such a result is, we think, open to grave doubt.

Cases 4, 7, 8, 12, 13, 15, 16, 19, 24, 26, 27, 28, 29, and 30 were all over ten years' standing, and had shown no symptoms since the disappearance of their secondaries. Some of them had taken rather irregular and intermittent treatment, but not enough, from our experience, to change their serologic reactions.

In fact, we do not think that any amount of treatment will ever get such cases negative if tested against cholesterinized antigens.

Cases, 1, 3, 5, 6, 17, 21, and 22 showed signs of an active syphilitic process at the time the serologic examination was made and represent among 500 known syphilitic cases, those where the diagnosis would have been seriously affected by dependence on plain antigen alone. This represents an advantage of 1.4 per cent in favor of cholesterinized antigen, but this slightly increased sensitiveness is more than offset by the high percentage of false positives which such antigens give.

The remaining cases, while free from symptoms, have all had more or less recent treatment, and are mostly cases of comparatively short standing, so that it is difficult to judge the relative value of the two antigens.

TABLE II

KNOWN SYPHILITIC CASES STRONGLY POSITIVE ONLY WITH CHOLESTERINIZED ANTIGEN

CASE NO.	ANTIGEN			HISTORY
	CHOLESTERINIZED 37.5° C.	PLAIN 37.5° C.	PLAIN 7-10° C.	
1	4*	0	0	Congenital—age 24 months. Treated since birth.
2	3	0	0	Infected 2 years ago. 3 doses of 606 and Hg at time. Treated up to 2 months ago. No symptoms at present.
3	4	0	0	Congenital lues, age 7 years. Never treated.
4	3	0	0	Infected 12 years ago. No symptoms in past 11 years. Treated off and on.
5	4	1	0	Infected 23 years ago. Treatment at time. Symptoms of beginning tabes.
6	3	0	0	Chancre 3 months ago. Malignant secondaries in mouth and throat and on body. Fever and marked debility.
7	4	0	0	Infected 17 years ago. Treated 2 years at time. Complains of pains in both knees.
8	4	0	0	Infected 12 years ago. ++++ W. R. with ice box method 3 months ago. Since then has had 4 doses of 606 and Hg to date. Latent.
9	3	0	0	Infected 9 years ago. Treated intermittently. Latent.
10	3	0	0	Infected 1 year ago. Hg and sod. cacodylate injections to date.
11	3	0	0	Date of infection not known. Tertiary ulcers 8 months ago. 4 doses 606 and Hg since.
12	3	0	0	Infected 23 years ago. Latent since. In past two years has taken 8 doses 606 and many injections of Hg without affecting Wass. reaction.
13	3	0	0	Infected 19 years ago. Treated at intervals since. No symptoms since secondaries.
14	3	0	0	Secondaries 1 year ago. Cleared up under 606 and Hg. Later developed iritis which cleared up under treatment. Husband is luetic. Latent.
15	4	0	0	Infected 18 years ago. Several secondaries. Treated at Hot Springs 3 months. No treatment since. Latent.

TABLE II (CONT'D)

CASE NO.	ANTIGEN			HISTORY
	CHOLESTERINIZED 37.5° C.	PLAIN 37.5° C.	PLAIN 7-10° C.	
16	4	0	0	Infected 11 years ago. Treated 2 years. No symptoms since. Spinal fluid is negative.
17	3	0	0	Infected 4 years ago. Treated by mouth for 3 years. Now has ulcer of septum.
18	3	0	0	Infected 8 years ago. Treated off and on since. 12 doses 606 and Hg in past 5 years. Latent past 7 years. W. R. with cholesterinized antigen has been 4 or 3 always.
19	4	0	0	Infected 17 years ago. Tertiary ulcers on both shins 10 years ago. Nothing since. No treatment in 10 years.
20	4	0	0	Secondaries 6 months ago. 6 doses 606 and Hg to one month ago.
21	4	0	1	Infected 10 years ago. Multiple subcutaneous gummata.
22	4	0	0	Infected 6 years ago. Little treatment. Has double iritis now.
23	4	0	0	Infected 7 years ago. 5 years ago took 6 doses of 606 and Hg for 3 months. No symptoms since secondaries.
24	4	0	2	Infected 12 years ago. Treated 3 years. No symptoms since. Spinal fluid is negative.
25	3	1	1	Infected 3 years ago. Several doses 606 and Hg intermittently to 6 months ago. Latent.
26	4	0	0	Infected 19 years ago. No symptoms since. 4 doses of 606 in past three months.
27	4	0	0	Infected 35 years ago. Irregular treatment. No symptoms since.
28	4	0	0	Infected 27 years ago. No symptoms since secondaries. No treatment in 20 years.
29	4	0	0	Infected 11 years ago. Severe secondaries. No symptoms or treatment in 10 years.
30	4	1	1	Infected 30 years ago. Latent since. No treatment since secondaries disappeared.

Note: The name 606 in these tables has been applied to all the various forms of salvarsan and neosalvarsan.

*4 = ++++, 3 = +++, 2 = ++, 1 = +, 0 = negative.

Taking up now the results obtained in the examination of 500 patients without history or symptoms of lues we obtained the figures shown in Table III.

Here is to be noted, not only the absence of false positive reactions with the ice box method, but also, as with the known cases of lues, the comparatively low percentage of doubtful reactions.

The one case in this group which gave a strong positive reaction with the ice box method was also positive with cholesterinized antigen and is No. 7 in Table IV, which gives in detail the histories of these cases showing false positive reactions.

This particular serum was from a man suffering from a rather severe anemia, apparently of a secondary type, who showed no improvement under mercury and

TABLE III

REACTIONS OBTAINED ON SERA OF 500 CLINICALLY NEGATIVE CASES

TEMPERATURE OF FIRST PHASE	TIME	ANTIGEN	POSITIVE		DOUBTFUL		NEGATIVE	
			No.	%	No.	%	No.	%
37.5° C.	1 hr.	Cholesterinized	19	3.8	33	6.6	448	89.6
37.5° C.	1 hr.	Plain	0	0	7	1.4	493	98.6
7-10° C.	4 hrs.	Plain	1	0.2	3	0.6	496	99.2

TABLE IV

NEGATIVE CASES GIVING POSITIVE REACTIONS WITH PLAIN AND CHOLESTERINIZED ANTIGENS

CASE NO.	ANTIGEN			HISTORY
	CHOLESTERINIZED 37.5° C.	PLAIN 37.5° C.	PLAIN 7-10° C.	
1	4	0	0	History negative. Dementia precox.
2	4	0	0	History negative. No symptoms.
3	4	0	0	History and examination negative. Has healthy six-week-old baby which gives a negative Wassermann reaction.
4	4	0	0	History negative. Has a severe acne.
5	4	0	0	History negative. Has a severe nephritis with albumin and casts.
6	4	0	0	Tuberculous glands of neck.
7	4	0	3	Anemia not improved under Hg and KI. Improved under iron and arsenic.
8	4	0	0	Morphine fiend. Mental symptoms clearing under treatment, for drug addiction.
9	4	0	0	Nine-year-old child with spina bifida. Father and mother healthy.
10	4	0	0	History and examination negative.
11	4	0	0	History and examination negative. Has wrenched back.
12	4	0	0	Chancroid 6 weeks ago. W. R. negative. 2 months later no clinical or serologic evidence of lues.
13	4	0	2	No symptoms. Father paretic. Mother healthy and has negative Wassermann.
14	4	0	0	History and examination negative. Has psoriasis.
15	3	0	0	History and examination negative. Healthy adult.
16	3	0	1	History negative. Has severe weeping eczema.
17	3	0	0	History and examination negative. Healthy adult.
18	3	0	0	History negative. Has Lichen planus.
19	3	2	0	History negative. Persistent headaches. Spinal fluid is negative.

potassium iodide but who picked up wonderfully under the administration of iron and arsenic. A very careful investigation failed to show any symptoms suggestive of lues, and the history, which we considered dependable, was absolutely negative. We feel, therefore, that this should be classed perhaps as a false positive reaction.

Among the cases in this group, positive only with cholesterin antigen, we feel that syphilis can be excluded with reasonable certainty. When such a positive reaction with cholesterin antigen only was found, a thorough and careful reinvestigation of the patient's history and clinical condition was instituted to determine whether any evidence of lues could be found.

The 113 patients whose serologic examinations are tabulated in Table V were classed as doubtfully syphilitic, and either gave a history or showed sypmtoms leading to such diagnosis.

TABLE V

REACTION OBTAINED ON SERA OF 113 CASES CLASSED AS DOUBTFULLY SYPHILITIC

TEMPERATURE OF FIRST PHASE	TIME	ANTIGEN	POSITIVE		DOUBTFUL		NEGATIVE	
			No.	%	No.	%	No.	%
37.5° C.	1 hr.	Cholesterinized	45	39.8	13	11.5	55	48.7
37.5° C.	1 hr.	Plain	4	3.5	9	7.9	100	88.5
7-10° C.	4 hrs.	Plain	8	7.0	8	7.0	97	86.0

The figures found in the examination of this class of cases are of little value, of course, in establishing the relative worth of the different methods of performing the Wassermann reaction, but there should be noted the wide divergence of the results obtained with cholesterinized antigen.

Personally, we feel that most of the cases strongly positive with plain antigen in incubator or ice box, represent syphilitic cases, but this belief could not be reconciled with the history and clinical findings as noted by the attending physicians.

Deducting the 7 per cent of strongly positive with plain antigen in the ice box from the 39.8 per cent of such positives found with cholesterinized antigen leaves 32.8 per cent more strongly positive reactions in this group when tested with cholesterinized heart extract.

Just why the two antigens should differ so widely among the patients of this group, while showing a difference of 6 per cent and 3.6 per cent, respectively, among the known syphilitics and known negative cases we can not explain, but judging from the figures obtained with the two known groups we would certainly feel that much more evidence than a positive Wassermann reaction performed only with cholesterinized antigen should be adduced before making a definite diagnosis of lues in the presence of doubtful histories and physical findings.

We have appended below a brief history of the patients in this group who gave serologic reactions of diagnostic strength.

CONCLUSIONS

1. Simple alcoholic heart extracts give the most reliable Wassermann reactions, provided the first phase of the reaction is carried out at 7° C. to 10° C.

2. A period of four to six hours at this temperature gives the best results. Longer periods (twelve to eighteen hours) may give doubtful or weak positive reactions.

3. We have found human heart extracts more dependable than beef or guinea pig heart preparations.

TABLE VI
DOUBTFUL CASES GIVING POSITIVE REACTIONS

CASE NO.	ANTIGEN			HISTORY
	CHOLESTERINIZED 37.5° C.	PLAIN 37.5° C.	PLAIN 7-10° C.	
1	4	0	0	Sore 3 months ago. Few spots on body later on. Slight general adenitis.
2	4	0	3	Suspected chancroid 10 years ago. No secondaries. Has itching, scaling palmar rash of both hands.
3	4	2	2	Husband luetic. Patient very nervous and irritable.
4	4	0	2	Scrofula when a child. Anemia and debility.
5	4	0	0	Father luetic. Has had 3 abortions. No clinical evidence of lues.
6	4	0	0	Indefinite. Has severe anemia with chronic nephritis.
7	4	0	0	Husband luetic. Has had chronic diarrhea which improved under antiluetic treatment.
8	4	0	0	Several sores at different times. History of doubtful rash which was probably acne.
9	2	0	4	No history. Prostitute, has general adenitis.
10	4	0	0	Sore 5 years ago. Low grade meningitis now. Spinal fluid not examined.
11	4	0	0	Prostitute. No history or symptoms.
12	4	0	0	No history. Psoriasis-like rash which has not improved under 606 and Hg.
13	4	0	2	Husband of Case 5. No history or symptoms except enlarged cervical glands.
14	4	0	0	Prostitute. No history or symptoms.
15	4	1	0	Prostitute. No history. Has eczema-like eruption on hands and feet.
16	4	3	2	No history. Underdeveloped boy of 16 years. Normal mentally. Parents apparently normal.
17	4	1	0	Sore 2 weeks ago. Appeared 3 weeks after exposure. General adenitis. Six weeks later sore healed. No clinical or serological change.
18	4	0	0	Sore 8 years ago. History of indefinite secondaries.
19	4	0	0	Gonorrhea 4 years ago. Now has persistent nocturnal headaches, not relieved by glasses. Spinal fluid negative.
20	4	0	0	Sore 1 year ago. Doubtful rash at time of sore. No symptoms now.
21	4	0	0	Prostitute. Denies primary. Had rash and sore throat six months ago. No clinical signs at present.
22	4	4	4	Prostitute. Denies primary or secondaries. Has ulcerated sore throat diagnosed as tbc, or lues.
23	1	1	4	Recent pregnancy. Baby healthy. History of recurrent abortions. Husband is luetic.

TABLE VI (CONT'D)

CASE NO.	ANTIGEN			HISTORY
	CHOLESTERINIZED 37.5° C.	PLAIN 37.5° C.	PLAIN 7-10° C.	
24	4	0	0	Prostitute. Denies primary or secondaries. Shows general adenitis. Has a few scars on tibia.
25	4	0	0	Prostitute. Denies history. Has an anemia which has improved under Hg and KI.
26	4	0	0	History negative. Paralysis of hands, feet and vocal cords. Spinal fluid is negative.
27	4	0	0	History negative. Has scaling palmar rash.
28	4	0	0	Sore 10 years ago. No secondaries. Small ulcer on leg 4 years ago. Cured with S. S. S.
29	4	4	3	Sore 4 years ago. Later developed rash diagnosed as Lichen planus. No symptoms at present.
30	4	0	1	Denies primary. Severe rash over body, face, and scalp 6 years ago, which disappeared under local treatment. No symptoms now.
31	4	0	0	Prostitute. Sore but no secondaries 1 year ago. 2 doses 606 at time of sore. No symptoms now.
32	3	0	0	Sore 7 years ago. No secondaries. Hg. by mouth for 18 months.
33	4	0	4	Denies primary. History of blood poisoning 16 years ago. No symptoms, but general health improved under specific treatment.
34	4	0	0	No history of primary or secondaries. Has chronic ulceration of throat.
35	3	0	0	Possible secondaries 2 years ago.
36	4	4	4	Denies primary and secondaries. Has syphilitic child.
37	3	0	0	Prostitute. Sore on two occasions. Denies secondaries.
38	4	0	0	Denies history of lues. Has copper-colored spots on shins and periostitis of both tibia.
39	4	0	0	Doubtful history of possible infection 20 years ago.
40	4	2	1	Sore 18 years ago. Diagnosed as chancroid. Has arthritis which does not improve under specific medication.
41	4	1	4	History doubtful. 1 abortion. 1 child died at 6 weeks with suspected lues. Husband insane.
42	4	0	1	Sore 1 year ago. Treated 3 months. Now has atypical rash on face and hands.
43	4	1	0	Sore 6 years ago. No secondaries. Has inequality of pupils, sluggish reflexes, and is very nervous.
44	3	0	0	Sore 3 months ago. 3 doses 606 and Hg at time. No treatment in 6 weeks.
45	3	0	0	Prostitute. Denies lues. Has periostitis of frontal bone.

4. Cholesterinized antigens even when used in small quantities will give false positive reactions in a considerable number of cases.

Recent experiments, which we have carried out and which we hope to report later, tend to show us that a fifteen-hour fixation time gives a slightly higher percentage of positive results than is obtained with a four-hour fixation time. No better results have been obtained by extending the fixation time up to twenty-four hours.

NOTE: Since this article was written, Ruediger: Jour. Infect. Dis., 1918, xxiii, 173; and Wile: Jour. Am. Med. Assn., 1919, lxxiii, 1526, have reported uniformly better results obtained with ice box fixation than with the older method of preliminary incubation at 37.5° C. Berghausen: Jour. Am. Med. Assn., 1919, lxiii, 996, likewise advocates ice box fixation, but states that he finds cholesterinized antigen very satisfactory and a fixation period of eighteen to twenty hours at 0° C. the best.

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A COMPARATIVE STUDY OF THE WASSERMANN TEST AND THE HECHT-WEINBERG-GRADWOHL MODIFICATION*

BY A. J. BLAIVAS, BROOKLYN, N. Y.

DURING the past few years many papers have been published describing and discussing the various modifications of the Wassermann test. Kolmer[1], in one of his publications, states his conclusions that the Hecht-Weinberg-Gradwohl reaction is superior to that of the Wassermann test. He found that 92 per cent of sera are satisfactory for the modified test, and that only a small percentage contains sufficient natural complement and amboceptor to hemolyze more than 0.5 c.c. of a 5 per cent sheep cell suspension. In routine work of the Hecht-Weinberg-Gradwohl test, therefore, larger doses of sheep cells are not required to determine the hemolytic indices of the sera.

The following are the results obtained by Kolmer. "In 7 per cent of the sera the hemolytic activity was too weak for the conduct of the Hecht-Weinberg-Gradwohl tests. Eighty-five per cent of the sera agreed with the Wassermann reaction so far as positive and negative were concerned. In 12 per cent, the Hecht-Weinberg-Gradwohl were positive and the Wassermann reaction negative with all antigens."

*From the Serological Laboratory of the Brooklyn Diagnostic Institute, Brooklyn, New York.

Kolmer[2] also claims that in every instance of a positive Hecht-Weinberg-Gradwohl modification, notwithstanding the negativeness of the Wassermann test, either the history or the clinical diagnosis, or both justified a diagnosis of syphilis, thus proving the superior delicacy of the Hecht-Weinberg-Gradwohl modification. The majority of sera he used were from known syphilitic persons under active treatment. However, in one to two per cent of the total number of cases the Hecht-Weinberg-Gradwohl tests were found NEGATIVE while the Wassermanns were POSITIVE. The sera of two of these patients were tested again about a week later, when a positive reaction was obtained in both the Wassermann and the Hecht-Weinberg-Gradwohl tests.

In another series of cases Kolmer mentions similar discrepancies between the Wassermann and Hecht-Weinberg-Gradwohl tests. In explanation, Kolmer suggests that the positive Wassermann and the negative Hecht-Weinberg-Gradwohl tests of the sera may have been due to "technical errors." In a later article, however, he says as follows:

"These results are quite similar to those previously reported in which the Hecht-Weinberg-Gradwohl and Wassermann reactions yielded similar results with 82 per cent of 362 sera. In the former series, I found six sera, or about two per cent yielding positive Wassermann and negative Hecht-Weinberg-Gradwohl reactions and in the present series, a similar result was observed. While in the former series two sera yielded pseudopositive or proteotropic reactions, none occurred in the present series, a result which I ascribe to the use of acceptable extracts of acetone-insoluble lipoids as antigens after titration with human instead of guinea pig complement."

In our own experiments, we have never met with a simultaneous positive Wassermann and negative Hecht-Weinberg-Gradwohl reaction. Such a contradiction in our opinion, is in all probability due to technical errors.

Regarding the reliability of the Hecht-Weinberg-Gradwohl test, Gradwohl[3] offers the following evidences:

"We found that in no case where we obtained a positive Hecht-Weinberg-Gradwohl and a negative Wassermann was our work at fault or the serologic diagnosis a surprise to the clinician. We must confess that there are certain cases of syphilis in which both tests were negative which were really syphilitic, as proved by true clinical manifestations and later the appearance of positive reactions in one or both tests at a subsequent examination. These were invariably cases of latent syphilis in robust individuals. We wish to add too, that the use of cholesterinized antigens does not measure up in accuracy to the use of the Hecht-Weinberg-Gradwohl test for syphilis."

It is my experience in over 10,000 comparative tests that many final readings in the Hecht-Weinberg-Gradwohl modification show a faint inhibition of hemolysis in tubes No. 12 and No. 13 or No. 13 only, the latter containing the largest amount of antigen.

Such reactions make a positive diagnosis of syphilis doubtful, unless the patient gives also a positive clinical history, or one permitting the assumption of the existence of a latent state.

TABLE I

	NAME	SEX*	WASSERMANN	H. I.**	H. W. G.***	REMARKS
1	K. C.	F	—	5	—	
2	B. S.	M	—	2	—	
3	J. D.	M	—	4	—	
4	E. S.	F	—	4	—	
5	K. M.	F	—	5	±	Faint inhibition of hemolysis in tube No. 13
6	J.A.P.	M	—	6	—	
7	D. S.	M	—	5	—	
8	S. G.	M	—	6	—	
9	G. G.	M	—	3	—	
10	I. L.	F	—	no index	—	
11	O. B.	M	—	5	±	Faint inhibition of hemolysis in tube No. 13
12	J. P.	M	—	no index	—	
13	P. C.	M	++++	no index	—	
14	M.W.	M	++++	6	+	Strongly positive ++++
15	E. W.	F	—	8	—	
16	S. H.	M	—	4	—	
17	A. B.	M	—	5	+	Positive in tubes 12 and 13
18	A. A.	M	—	2	—	
19	E. K.	M	—	3	±	Faint inhibition of hemolysis in tube No. 13
20	M.L.	M	—	1	±	Faint inhibition of hemolysis in tube No. 13
21	O. L.	M	—	3	—	
22	G. H.	M	—	2	—	
23	T. F.	M	— —	5	+	Early locomotor ataxia
24	J. F.	M	—	1	—	
25	M. G.	M	—	3	—	
26	G. K.	M	—	5	—	Nephritis
27	A. V.	F	±	8	+	
28	T. K.	F	—	5	—	
29	Dr. G	M	—	no index	—	Nephritis
30	M. H.	F	—	4	—	
31	R. C.	F	++	5	+	Known case of syphilis
32	J. R.	F	++++	5	+	Patient's husband has paresis
33	M. M.	M	—	no index	—	
34	J. M.	F	±	2	+	
35	A. V.	M	—	7	—	
36	C. V.	M	—	6	—	
37	T. F.	M	++	7	+	H. W. G. strongly positive ++++
38	G. O.	M	—	4	—	
39	D. J.	M	±	8	+	Treated case (1 injection)
40	D. J.	M	—	6	—	Treated case (1 injection)
41	G. T.	F	—	5	—	
42	P. H.	M	—	no index	—	
43	G.J.G.	M	—	9	—	
44	F. K.	M	—	7	—	
45	K. M.	F	—	no index	—	
46	J. P.	M	—	2	—	
47	R. L.	F	—	5	—	
48	S. F.	M	—	2	—	Treated case
49	A. A.	M	—	4	—	Locomotor ataxia
50	J. C.	F	—	3	+	H. W. G. strongly positive ++++
51	R. M.	F	—	2	—	
52	J. S.	M	—	3	—	Treated case (2 injections)
53	S. S.	F	—	2	—	
54	D. K.	F	+++	5	+	Pregnant woman. Syphilis known
55	F. J.	M	—	4	—	
56	M. S.	M	—	8	—	
57	J. K.	M	—	7	—	
58	N. T.	M	—	6	—	Arthritis

*F. Female. M. Male. **H. I. Hemolytic Index. ***H. W. G. Hecht-Weinberg-Gradwohl.

TABLE I (CONT'D)

	NAME	SEX*	WASSERMANN	H. I.**	H. W. G.***	REMARKS
59	M. A.	F	—	4	+	Weakly positive (in tube 13)
60	C. M.	F	—	4	—	
61	R. M.	F	—	7	—	
62	C. W.	M	—	no index		
63	K. M.	F	—	3	+	Weakly positive (in tube 13), see Case 5
64	H. S.	F	—	5	—	
65	J. S.	M	—	4	—	Treated case (3 injections)
66	S. F.	M	++++	2	+	Known case of syphilis
67	M. D.	M	—	3	—	
68	M.W.	F	—	3	—	Pregnant
69	W. W.	F	—	no index		
70	N. N.	M	—	no index		See Case 5
71	K. M.	F	—	4	+	Inhibition in 12 and 13
72	O. M	M	—	4	+	Inhibition in 12 and 13
73	C. Z.	M	—	no index		
74	J. B.	M	—	1	—	
75	F. S.	M	—	no index		
76	S. S.	F	—	2	+	Inhibition in 12 and 13
77	J.E.B.	F	—	5	—	
78	F. D.	M	—	2	+	Inhibition in 13
79	J. A.	M	—	3	—	
80	T. F.	M	+	2	+	Early locomotor
81	M. R.	F	—	no index		
82	E. M.	M	—	2	—	
83	M. A.	F	—	3	+	Inhibition in 13
84	B. J.	F	—	2	+	Inhibition in 12 and 13. Pregnant woman
85	M. K.	F	—	no index		
86	M. P.	M	—	no index		
87	M. W.	F	—	3	—	
88	Dr. B.	M	—	3	—	
89	M. M.	F	—	4	—	
90	R. W.	F	—	5	—	
91	G. E.	M	—	3	—	
92	M. M.	F	—	4	—	
93	E. S.	F	—	2	—	
94	B. J.	F	—	1	—	2 days after delivery
95	L.A.T.	—	—	2	—	
96	S. N.	F	—	no index		
97	A. B.	F	—	3	—	Pregnant
98	F.A.B.	F	—	2	+	Very faint inhibition in No. 13
99	T. F.	M	—	2	—	Early locomotor
100	D. T.	—	—	no index		

*F. Female. M. Male. **H. I. Hemolytic Index. ***H. W. G. Hecht-Weinberg-Gradwohl.

Gradwohl claims that 98 per cent of the sera contain natural antisheep amboceptor. This may be true perhaps if the patient's blood is examined shortly after withdrawal or, at the latest, within a period of twenty-four hours. If correct, then it would necessitate working with the Wassermann test in the ordinary laboratory daily. My experience, however, does not seem to coincide with the statement presented by Gradwohl, as of the 100 cases shown in Table I, 17 per cent contained either no natural complement or antisheep amboceptor or neither of the two.

The average hemolytic index in our series was four, i. e., 0.1 c.c. of serum contained enough natural complement and amboceptor to hemolyze 0.4 c.c. of a 5 per cent suspension of sheep cells. The technic used in the Hecht-Weinberg-

Gradwohl test was exactly the same as done by Gradwohl in his laboratories which is as follows:

Place 0.1 c.c. of unheated serum into each of the fourteen test tubes which are placed in the special rack as shown in Fig. 1. Put 1 c.c. of normal salt in Tube 1, 0.9 c.c. in Tube 2, 0.8 c.c. in Tube 3, 0.7 c.c. in Tube 4, 0.6 c.c. in Tube 5, 0.5 c.c. in Tube 6, 0.4 c.c. in Tube 7, 0.3 c.c. in Tube 8, 0.2 c.c. in Tube 9, 0.1 c.c. in Tube 10, 0.2 c.c. in Tube 11, 0.15 c.c. in Tube 12, 0.1 c.c. in Tube 13, 0.3 c.c. in Tube 14. The first ten tubes are used to determine the hemolytic index of the blood under examination. In other words, the object is to find the amount of natural complement and amboceptor in the suspected serum. With Tubes 11, 12 and 13 the actual test is carried out. Tube 14 is the serum control tube. Now add 0.1 c.c. of a 5 per cent suspension of sheep cells to Tube 1, 0.2 c.c. to Tube 2, 0.3 c.c. to Tube 3, 0.4 c.c. to Tube 4, 0.5 c.c. to Tube 5, 0.6 c.c. to Tube 6,

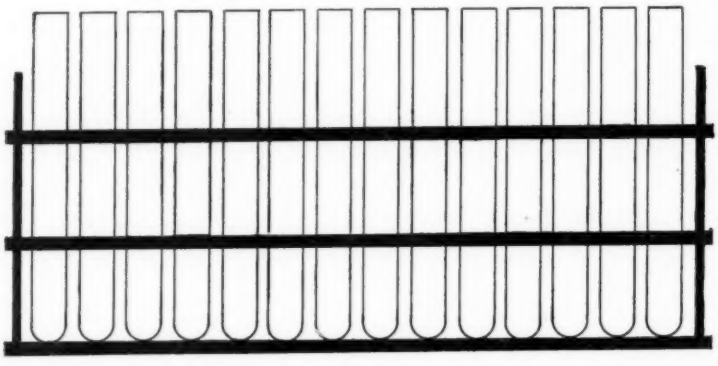

Fig. 1.

0.7 c.c. to Tube 7, 0.8 c.c. to Tube 8, 0.9 c.c. to Tube 9 and 1.0 c.c. to Tube 10. Place 0.1 c.c. of titrated ether-soluble, acetone-insoluble antigen into Tube 11, 0.15 c.c. of the antigen into Tube 12 and 0.2 c.c. of the antigen into Tube 13. These three tubes (11, 12, and 13) are the tubes in which the final readings are made and for that reason the antigen is added to them alone. Tube 14 being the serum control tube, received, of course, NO antigen. The tubes are now well shaken and placed into a water-bath for one-half hour at 37.5°C. At the end of this time the rack is removed from the water-bath and the last tube which shows complete hemolysis is the hemolytic index. For example, if Tube 5 is the last where complete hemolysis occurs, the hemolytic index is five. This means that 0.1 c.c. of the serum will hemolyze 0.5 c.c. of a 5 per cent suspension of sheep cells. In order to determine the amount of sheep cells to put into Tubes 11, 12, 13 and 14, Gradwohl has worked out the following table:

If the hemolytic index is from,

1 to 4 add 0.1 c.c. of sheep cells to the last four tubes.

5 to 7 add 0.15 c.c. of sheep cells to the last four tubes.

8 to 10 add 0.2 c.c. of sheep cells to the last four tubes.

After the addition of the sheep corpuscles to the last four tubes, the contents of the tubes are well shaken and again placed in the water-bath for one-half

hour at 37.5° C. The rack is then removed and the results read just as in the Wassermann test.

Of the 19 cases which showed a positive or a borderline Hecht-Weinberg-Gradwohl test and a negative or a borderline Wassermann test, we have been able to obtain only 14 histories. In the other 5 cases data relating to luetic infection were unobtainable inasmuch as they were cases either referred special diagnosis or for special reasons were not questioned in that direction.

In most of the 14 cases in which a discrepancy between the Wassermann and its modification was found, there were direct evidences either of an early infection, or of a mild easily overlooked case of syphilis or of a syphilitic association or consanguinity. Perhaps a short resume of the histories of these cases, in which a negative or borderline Wassermann and a positive or suspicious Hecht-Weinberg-Gradwohl test were obtained might not be amiss.

CASE No. 5.—K. M., American, age 31, female. Negative personal history. The patient's husband contracted lues about fifteen years ago, with secondary but no tertiary manifestations later. Has two perfectly healthy children, but soon after last confinement, patient developed an eruption on the dorsal surfaces on the upper and lower extremities, copper-colored, circular, giving no subjective symptoms. The Wassermann negative, the Hecht-Weinberg-Gradwohl reaction positive in tubes 13 and 14. Six-tenths of a gram of salvarsan was given intravenously, after which the eruption disappeared completely, also the headaches of which the patient complained previous to the injection.

CASE No. 21.—A. V., Russian, age 32, female. Negative family history. At the age of eight the patient had an unknown arthritic affection. Patient is married ten years and has had *four miscarriages*, and finally had one living child. Had nasal troubles for many years and was in a hospital eleven years ago for some abscess, the character of which is not related. Patient has had intensive treatment in a hospital and also privately with salvarsan, mercury, and iodides. This case seems to serve as a good illustration of the superiority of the Hecht-Weinberg-Gradwohl reaction over the Wassermann test, inasmuch as it represents a known and treated case of syphilis, giving a negative Wassermann but still showing a positive in the Hecht-Weinberg-Gradwohl test.

CASE No. 39.—D. J., Italian, age 32, laborer, male. The patient presents himself with a typical chancre of the right index finger. A dark-field microscopic examination proved the presence of Spirochete pallida. The patient, skeptical of the diagnosis first, refused the use of salvarsan, but returned several weeks later for a blood test. The Wassermann and the Hecht-Weinberg-Gradwohl tests disclosed a four-plus positive reaction. After the injection of salvarsan, the Wassermann showed a borderline reaction and the Hecht-Weinberg-Gradwohl still remained strongly positive, illustrating again the higher qualifications of the Hecht-Weinberg-Gradwohl test over the Wassermann reaction. After further injections of salvarsan, both the Wassermann and the Hecht-Weinberg-Gradwohl tests cleared up.

CASE No. 59.—M. A., Italian, age 28, female. Patient was married nine years ago, but has never become pregnant. Had her left ovary removed four years ago. The patient had an iritis, also an enlarged right axillary gland. The Wassermann test in August, 1918, showed a two-plus positive, but the examination at the present time was negative. The gonorrheal complement-fixation test also showed a two-plus positive reaction. This case serves as another good example where the Wassermann becomes negative at an earlier date than the Hecht-Weinberg-Gradwohl test.

CASE No. 65.—J. S., Italian, age 28, longshoreman, male. The patient gave clinical evidences of syphilis and also a history of having a chancre and secondary signs. The Wassermann and the Hecht-Weinberg-Gradwohl tests were both four-plus positive. After injections of salvarsan, the Wassermann and Hecht-Weinberg-Gradwohl tests became negative. At a later date another blood test was made and a negative Wassermann and a

strongly positive Hecht-Weinberg-Gradwohl reaction was obtained. The patient, however, felt perfectly well and refused further treatment. The fact, though, remained that the modified test reappeared earlier than the Wassermann did.

CASE No. 72.—O. M., American, age 33, salesman, male. Patient had a chancre thirteen years ago with following secondary eruptions. Had undergone intensive syphilitic treatment. The last injection of salvarsan was administered about a year and a half ago. He is married five years and has two healthy children. His wife (Case 5) was treated for secondary eruptions six months ago at which time she was nine months after her last pregnancy. This is a case of undoubted previous infection.

CASE No. 78.—F. D., Italian, age 36, laborer, male. Contracted syphilis nine years ago. Patient is married. His wife had been pregnant three times, but has no living child. His blood gave a four-plus Wassermann reaction four years ago. One year ago, it showed one plus. The patient has never had any salvarsan injections, but has received 100 intramuscular mercurial treatments. For one year treatment was stopped. The following year the patient received 24 intramuscular injections of mercury. the last one two weeks before the blood test was made. This is another undoubted case of syphilis, and illustrates again how the Hecht-Weinberg-Gradwohl modification is superior to the Wassermann test.

CASE No. 76.—S. S., Austrian, age 40, female. Married 23 years and has four children living and well. No miscarriages. Mother died of cancer. Patient was operated for uterine tumor four years ago. She complains of attacks of acute pains in the heart region, cyanosis, faint feeling, and palpitation. The attacks occur mostly at night. The patient is inclined to be obese and is mentally very apathetic. She shows a moderate exophthalmos and Mobius in the left eye. The pupils react sluggishly to light, and her reflexes are exaggerated. In this particular case, lues as the basis of the above symptoms can not be excluded, and it may therefore be counted as very suspicious. Perhaps the future will disclose further additional data to justify the diagnosis of latent lues, which makes it necessary to have further blood tests made.

CASE No. 83.—M. A., Irish, age 29, unmarried, female. Patient was admitted to the Prospect Heights Hospital, Maternity Ward, to be confined with a second child; both children are living and well. She denies syphilitic infection. Her mother died of asthma, her father is living, is a habitual drinker and has heart trouble. Her four brothers and three sisters are living and well. She complains of severe headaches and backaches. She also presents a positive Romberg, and clinically seems to be very suspicious of syphilis. She refused a recital of her previous history and also refused to submit to physical examination. Perhaps promiscuous intercourse may have caused mild infection of syphilitic nature and thus the cause of the positive Hecht-Weinberg-Gradwohl test.

CASE No. 84.—B. J., American, age 20, unmarried, female. This is the *girl's* first childbirth, both her parents are alive and well, also the rest of her family. Patient denies syphilitic infection or previous abortions. She has suffered fainting spells about once a week, from her seventeenth year. She also complains of severe headaches. Such a case deserves further watching, perhaps she may have had a syphilitic infection which she either denies or does not know of. She also refused a physical examination.

CASE No. 17.—A. B., Russian Hebrew, age 38, male. Married nine and a half years and has three healthy children. Suffers from pulmonary phthisis. Family history negative. He gives no history of venereal disease, and shows no clinical evidences of syphilis. This case may be classed as *very* doubtful.

CASE No. 11.—O. B., Russian, age 24, female. Venereal history negative. Married four years and has two children. No venereal history in husband who is, however, an excessive drinker. Shows no clinical manifestations, either objective or subjective of lues. Admits also of having had some throat troubles soon after marriage and never before. Patient now suffers from achylia gastrica without any pathologic basis as proved by a very careful clinical and laboratory analysis. This case permits suspicion of an early infection with secondary manifestations in the throat. Nevertheless the physician should not make a diagnosis of syphilis unless further proof is obtained.

CASE No. 50.—J. C., Italian, age 54, female. Married 34 years, pregnant fifteen times and seven were miscarriages. Patient has been ailing for the past seven years and has aches all over the left half of the body, with headaches either on the top or the left side. Also suffers from lack of weight. Shows gastrointestinal symptoms due to gall bladder disease. A physical examination disclosed a very sluggish left pupil, an appreciable enlargement of the left lobe of the liver and very highly increased reflexes. This case presents sufficient data permitting the assumption of a possible latent syphilis, in spite of the absence of definite data regarding such a state from the patient's history.

CASE No. 19.—E. K., Hungarian, age 40, baker, male. The patient had gonorrhea at 20, now complains of backaches, pain in pectoral region, numbness of extremities, pains in back of head, and noises in the right ear. Patient is also constipated and has an enlarged prostate. There seemed to be no history or physical signs of syphilis. A positive diagnosis of syphilis on the grounds that the blood of this patient showed a positive in the Hecht-Weinberg-Gradwohl modification is not therefore justifiable. This case, therefore, must also be classed as *very* doubtful.

In the aforegoing histories something suspicious was found in every case which gave either a positive or borderline reaction in the Hecht-Weinberg-Gradwohl test except two (see Cases 17 and 19). Nevertheless, in my opinion the Hecht-Weinberg-Gradwohl test should *never* be used alone, but only in conjunction with the Wassermann test, as at the present time there is not sufficient data available to justify its independent use and reliability. It is also unfortunate that some sera do not contain any natural complement. With such bloods the Hecht-Weinberg-Gradwohl test can not be used at all. In bloods that have no hemolytic index, we must depend upon the Wassermann test alone. The clinician must also exercise caution in diagnosing a case as syphilis when the Hecht-Weinberg-Gradwohl test *alone* is positive, while the Wassermann test is negative. When such results are obtained, a very careful clinical history must be taken and a very careful physical examination made before a diagnosis is pronounced.

SUMMARY

The following are the results with 100 comparative tests:

The above cases were not selected ones, but cases applying to the Brooklyn Diagnostic Institute for general diagnosis.

1. In 65 per cent of the sera the Wassermann and the Hecht-Weinberg-Gradwohl tests were either positives or negatives in both tests.

2. Nineteen per cent of the sera showed a positive or borderline in the Hecht-Weinberg-Gradwohl test and a one-plus or a borderline in the Wassermann reaction.

3. Seventeen per cent had no hemolytic index. (Of course in these cases the Hecht-Weinberg-Gradwohl test could not be employed.)

4. Five per cent showed a strong positive in the Hecht-Weinberg-Gradwohl test, and a negative in the Wassermann.

5. Five per cent showed a positive in Tubes 12 and 13 in the Hecht-Weinberg-Gradwohl test and negative in the Wassermann.

6. Four per cent showed a positive in Tube 13 in the Hecht-Weinberg-Gradwohl test and a negative in the Wassermann.

7. Two per cent showed very doubtful reactions. (See cases 17 and 19.)

8. Our average hemolytic index was four, not including those sera that had no index at all.

Gradwohl claims that only 2 per cent of the sera lack enough natural complement for the Hecht-Weinberg-Gradwohl test. This may be the case if the test is made on fresh sera. As already mentioned our tests were performed three times a week, so that some of the sera were about 48 hours old. Perhaps this may serve as an explanation of the 17 per cent that had no hemolytic index. We are now making the tests on the day the blood is withdrawn so as to determine what per cent of the sera lack enough natural complement to perform the Hecht-Weinberg-Gradwohl test under those circumstances.

Gradwohl claims that in the Hecht-Weinberg-Gradwohl test, a complete inhibition of hemolysis is obtained and states it thus:

"Furthermore, I wish to be understood as stating, that when I speak of negative Wassermann and positive Hecht-Weinberg-Gradwohls, we do not mean a faint inhibition of the latter and a complete hemolysis in the former. The results are astonishingly different and striking, on the one hand, a clean cut negative Wassermann, on the other a definite and distinct positive Hecht-Weinberg-Gradwohl."

My experiences with the Hecht-Weinberg-Gradwohl test, not only in this series herein reported, but also of former work, permit me to state that the Hecht-Weinberg-Gradwohl gives not only a faint inhibition of hemolysis, but sometimes a complete hemolysis in Tubes 11 and 12 and a complete or faint inhibition of hemolysis in Tube 13. It may also give a complete hemolysis in Tube 11 and a faint or complete inhibition in Tubes 12 and 13, and finally it may give a faint inhibition in Tubes 11, 12, and 13. Any of these reactions would be termed borderline reactions.

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A METHOD FOR PREPARING BACTERIOLOGIC MEDIA CONTAINING ASCITES FLUID*

By Linwood G. Grace, D.D.S., Cleveland, Ohio

THE use of broth to which certain amounts of ascites fluid are added has been advocated for some time for cultivating streptococci and other organisms. The usual directions given for its preparation are that the sterile fluid be added to the broth which has been previously made and sterilized. The method requires most careful attention to details and offers numerous sources for contamination. If the fluid has not been collected aseptically, it can, of course, be sterilized by passing through an unglazed porcelain filter. There still remains, however, the problem of getting it into tubes of broth in definite quantities without contamination. H. Bierry (Comptes Rendus des Seames de la Société de Biologie, 1916, No. 7, p. 270) called attention to the fact that ascites and other albuminous fluids may be autoclaved at 112° C. without coagulating, if previously rendered alkaline. It may then be neutralized and reautoclaved and still remain clear. Or it may be added to broth or agar, the reaction of which is adjusted as required, and autoclaved without causing coagulation or precipitation in the media.

It is quite obvious that such a process infinitely simplifies the preparation of such a media. The ascites can be added to the broth or agar in definite amounts before it is tubed. This is far more accurate than adding it to the media after it has been tubed and also less laborious.

In order to determine the minimum amount of alkali necessary to add to ascites fluid to prevent coagulation when autoclaved, we carried out the following experiment:

The sample of ascites on hand was nearly neutral. Phenolphthalein was used as the indicator. Twenty test tubes were selected and into each test tube were placed 10 c.c. of ascites fluid. Definite amounts of alkali were added to each tube and all autoclaved at 15 pounds pressure, 118° C., for 15 minutes. This is slightly higher than the temperature suggested by Bierry, but is the standard we have used for some time for autoclaving media. After autoclaving, all those which were still liquid were filtered. The results are noted in the accompanying table, together with the amounts of alkali added to each tube. A separate tube in which no alkali was added was run as a control. As a final test a few drops of a normal acid solution were added to each tube. A flocculent precipitate was thrown down in each case upon the addition of the first drop of acid.

From the table it would appear that six or seven drops of 40 per cent NaOH to each 10 c.c. of ascites, or about 2½ per cent alkali, gave the best results. However, before adding the alkali, the ascites should be titrated, as a more acid fluid would undoubtedly need a greater amount of alkali to produce the same results. It is better to add small quantities of a strong alkali than large quantities of a weak one on account of diluting the fluid.

*From the Laboratories of the Research Institute of the National Dental Association, Cleveland, Ohio.

When the ascites is received it is rendered alkaline by adding 2½ per cent of 40 per cent NaOH, autoclaved 15 pounds at 118° C. for 15 minutes and stored in the ice box until ready for use. When the media is made up this ascites is added in the required amounts, usually 5 per cent, the reaction of the entire batch adjusted, filtered, tubed, and autoclaved 15 pounds at 118° C. for 15 minutes. We have been using this method for over a year, and results have shown that all organisms tested grow equally as well as in media prepared by the old method.

TUBE NO.	AMOUNT OF ALKALI ADDED	APPEARANCE AFTER AUTOCLAVING	APPEARANCE AFTER FILTERING
1	0.1 c.c. N/1 NaOH	Entirely coagulated. Appear almost the same as tube without alkali	Not filtered
2	0.2 c.c. N/1 NaOH	Nearly all coagulated. Very little liquid left	Not filtered
3	0.3 c.c. N/1 NaOH	Same as 2	Not filtered
4	0.4 c.c. N/1 NaOH	Partly coagulated. Coagulum in suspension	Same as before filtering
5	0.5 c.c. N/1 NaOH	Same as 4	Same
6	0.6 c.c. N/1 NaOH	Same as 4	Same
7	0.7 c.c. N/1 NaOH	Same as 4	Same
8	0.8 c.c. N/1 NaOH	Same general appearance as 4. Slightly less coagulum	Same
9	0.9 c.c. N/1 NaOH	Same as 8	Same
10	1.0 c.c. N/1 NaOH	Same as 8	Same
11	1 drop 40% NaOH*	Nearly all coagulated	Not filtered
12	2 drops 40% NaOH	Partly coagulated. Coagulum in suspension	Same as before filtering
13	3 drops 40% NaOH	Same as 12	Same
14	4 drops 40% NaOH	Very little coagulation. Precipitate at bottom of tube	Cleared a little
15	5 drops 40% NaOH	Very little if any coagulation. Precipitate at bottom of tube	Fairly clear
16	6 drops 40% NaOH	Almost entirely clear. Precipitate at bottom of tube	Clear
17	7 drops 40% NaOH	Same as 16	Clear
18	8 drops 40% NaOH	Not as clear as 16	Not as clear as 16
19	9 drops 40% NaOH	Same as 18	Same
20	10 drops 40% NaOH	Same as 18	Same

*The pipette used to drop the 40% NaOH dropped about 0.04 c.c. to a drop, as nearly as could be measured.

THE EARLY DIAGNOSIS OF TYPHOID AND PARATYPHOID INFECTIONS*

By Henry J. Goeckel, Ph.D., Plainfield, N. J.

THE extensive employment of typhoid and paratyphoid vaccine for immunization is liable to render the Widal agglutination reaction on the blood serum as usually employed of doubtful value in many cases of suspected typhoid infection.

While blood cultures may prove a valuable aid in such cases and in others before a typical Widal reaction is obtainable, the method is time-consuming and it is neither convenient nor a desirable procedure in many cases.

I have recently had several cases of suspected typhoid infection referred to the laboratory, which either failed to give the agglutination reaction for typhoid and paratyphoid bacilli or gave only a partial agglutination of doubtful diagnostic value.†

In the first case which led to the investigation incorporated in this paper, the blood serum failed to show any agglutination. On the very day that the Widal reaction was made, the pathologic interne, while making the urine examinations, noted a moderate number of pus cells and numerous motile rod bacteria in the urine from this case. The specimen had been obtained at least four hours before the blood. As it was not secured aseptically, another one was obtained with a sterile catheter directly into a sterile container. This urine likewise showed pus and numerous motile rod bacteria.

Upon subjecting the lightly centrifuged bacterial suspension (without washing) to a series of agglutination tests, they proved to be paratyphoid bacilli, Type B.

The agglutinating sera employed had the following titer: typhoid, 1-4000; paratyphoid Types A and B, each 1-10,000; coli, 1-2000. They were employed in the following dilutions; Typhoid, 1-2000; paratyphoid A and B, each in 1-5000, and the coli serum in 1-1000 dilution. All gave an entirely negative test except the paratyphoid B serum.

The blood count showed 73% polymorphonuclear neutrophile leucocytes, 22.6% lymphocytes, and 4.4% mononuclear leucocytes.

The temperature was 104.2° F., the highest attained until discharged.

The urine showed a distinct trace of albumin, moderate indican test, moderate pus, few oxalate crystals and numerous motile rod bacteria.

The very next day a case was sent into the hospital for observation as a suspected case of appendicitis.

Child, 4 years, 11 months, 9/24/19. The day of admission the maximum temperature was 101.8° F. dropping to 99.9° F. The white cell count showed 17,000 cells per c.mm. of which 76% were polynuclear leucocytes and 24% mononuclear cells.

The Widal reaction on the blood showed partial agglutination for typhoid only in the 1-20 dilution. A catheterized urine as in the preceding case showed a moderate number of

*From the Clinical and Pathological Laboratory of Muhlenberg Hospital, Plainfield, N. J.

†Suspensions of live bacilli are employed by the hanging drop microscopic method. Agglutination and inhibition of motility are both noted in dilutions of 1-20 to 1-120.

isolated leucocytes and a moderate number of motile rod bacteria. These when tested with agglutinating sera of the same dilutions as in the preceding case, proved to be typhoid bacilli.

9/25/19.—Widal reaction on blood agglutinated to 1-60 dilution.

9/28/19.—Highest temperature was attained, 103.8° F.

10/1/19.—Widal reaction on blood agglutinated to 1-120 dilution.

10/3/19.—W. C. C. 18,000; polynuclear leucocytes 52%; mononuclear cells 45%; eosinophiles 3%.

10/5/19.—Routine urine examination was negative.

In the third case in the series the data given was that a month previous, the patient (a boy of 10 years) showed a temperature of 104° F. At the same time the lower right abdominal quadrant proved tense and tender. Upon consultation it was decided to remove the appendix which was found to be moderately inflamed. The case apparently made an uneventful recovery only to show the same temperature elevation four weeks later without any apparent physical distress, etc.

10/3/19.—Blood count showed 5,800 white cells of which 45.5% were polynuclears; 39% lymphocytes; and 15.5% were mononuclear leucocytes.

10/4/19.—Widal reaction positive for typhoid. On the same day the urine showed a moderate number of motile rod bacteria, no pus and no casts. The bacteria were concentrated by centrifuging at high speed. Most of the supernatant urine was decanted, and the residue was thoroughly agitated to break up any possible clumps resulting from the centrifuging. When subjected to a series of agglutination tests as were the preceding cases, they proved to be typhoid bacilli.

SUMMARY

In the first case while the Widal reaction and the blood count were in no way indicative of a typhoid or paratyphoid infection on the day of examination, the paratyphoid bacilli were identified in the urine.

In the second case the blood count was likewise of no value, in fact it was somewhat suggestive of a leucocytosis. The Widal reaction was doubtful, while the bacilli in the urine were identified as typhoid bacilli seven days before a typical Widal reaction was obtained and nine days before the blood count was typical.

In the third case the bacilli were identified at the same time that the blood count and Widal reaction showed typical for typhoid.

CONCLUSIONS

Although it is stated that the typhoid and paratyphoid bacilli appear in the urine some time after the second week of infection, the cases examined showed:

1. That it is possible to obtain and identify them in the urine by agglutinins before the blood shows a positive Widal reaction or a typical cell count.

2. By this means a prompter report was given than if blood culture had been resorted to.

3. It is a more definite method of identification of the infection than is identifying agglutinins in the patient's blood serum.

4. It is more positive and eliminates reliance on the Widal reaction on blood serum.

5. It should be resorted to whenever possible in patients who may have a natural or acquired agglutinating capacity due to previous infection or through the use of vaccines.

HEXAMETHYLENAMINE INTERFERES WITH THE TEST FOR INDOXYL IN URINE*

By Henry J. Goeckel, Ph.D., Plainfield, N. J.

KNOWING that formaldehyde when added to urine prevents the Obermeyer hydrochloric acid-ferric chloride reagent from reducing the indoxyl compounds to indican, I wish to call to the attention of those using this reagent that the administration of hexamethylenamine (i.e., urotropin, formin, etc.,) likewise prevents the reduction to indican.

This can be easily demonstrated by taking a urine high in indoxyl content, mixing with it some urine from a person taking hexamethylenamine, and applying the test.

The result will be a negative or very slight indican production while a control with an equal volume of the original urine will give a pronounced indican test.

DEVICE FOR WITHDRAWING BLOOD FROM VEINS

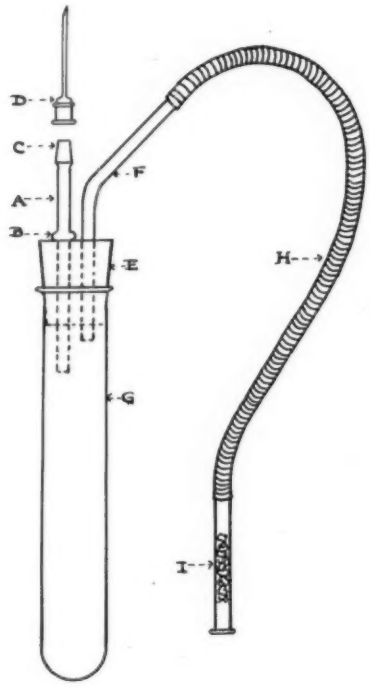
By Clyde L. Cummer, M.D., Cleveland, Ohio

A NUMBER of devices have been suggested for the withdrawal of blood from the veins for serologic, bacteriologic or chemical examination. The instrument long in vogue and still extensively utilized is the syringe. With a well made Luer syringe excellent service is obtained, since the use of washers or packing about the piston is obviated. The chief drawback is the expense, which is especially an item if much work is being done. Unfortunately, the vacuum in certain makes is often far from perfect. A needle is used by many for obtaining blood for the Wassermann reaction without the employment of negative pressure. This is reasonably satisfactory for serologic or chemical work, but is slow and can not be employed when cultures are to be made, on account of the likelihood of contamination. A larger needle is needed than is required when vacuum is utilized. While the Keidel tube is well adapted for serologic work and may be modified for bacteriologic work, it represents a definite expense, since it can not be resterilized and used again. In the writer's opinion the most practical instrument is the MacRae needle, which has been on the market for a number of years. It is, however, made of steel and corrodes readily. When it is frequently employed, the needle point is likely to lose its temper and the cannula portion so weakened by rust that it breaks off when this accident is least expected and least desired. Stitt has devised an extremely simple apparatus, employing a similar principle, made of glass, a piece of rubber tubing connecting the glass tubing which serves as an inlet to the needle. This is a disadvantage, since one hand

*From the Clinical and Pathological Laboratory of Muhlenberg Hospital, Plainfield, N. J.

must be used to introduce the needle and one to hold the apparatus. We have attempted to combine the good features of the MacRae and the Stitt outfits in an apparatus which is illustrated in the sketch.

A is a piece of glass tubing the distal tip (*C*) of which is ground to receive the Luer needle (*D*) by slip-joint connection. A collar or flange is blown into the tubing at the point (*B*) to prevent the tube from slipping down through the opening to the rubber stopper (*E*). A second piece of glass tubing (*F*) of approximately the same bore is bent slightly at an angle at one end, is introduced into a second hole in the rubber stopper (*E*). The lower end of the glass tube (*A*) should project about one-half inch or more below the bottom of the rubber stopper than does the lower end of glass tubing (*F*). To the upper end of glass tubing (*F*) is attached a piece of rubber tubing (*H*) about six inches long into



whose distal end is inserted a section of glass tubing (*I*) which is stuffed lightly with cotton and serves as a mouthpiece. The rubber stopper (*E*) should be such a size that it will fit snugly into the mouth of the test tube, flask or bottle which is to be employed for receiving the specimen.

With this apparatus a vacuum is secured by exhaustion of the air from the tube or flask by the operator, who sucks out the air through the rubber tubing. There is no danger of blood being drawn into the operator's mouth if the lower orifice of the tube (*F*) is kept above the lower orifice of the tube (*A*) when the apparatus is in use. Further security on this score is given by the cotton stopper in the mouthpiece. The entire apparatus may be handled with one hand, the tube serving as a handle, the rest of the outfit, except for the rubber tubing, forming a rigid unit. The other hand is left free for handling the tourniquet, steadying

the patient's arm, etc. There are no parts to rust or corrode, except the needle. Any size Luer needle may be used, which is of distinct advantage. For bacteriologic or chemical work, the outfit may be sterilized by boiling, while if it is desired to use the outfit for blood cultures, it may be wrapped in cloth and sterilized in the autoclave. The apparatus is convenient for bacteriologic work since the blood may be drawn directly from the vein without contact with outside atmosphere into a container charged with sodium citrate or with citrated physiologic salt solution, stoppered with a sterile stopper, and taken to the laboratory, where cultures may be made as desired. Cleaning is readily effected by inserting the stopper into the outlet of the cold water faucet and letting the water run through.

The apparatus has been made for me through the cooperation of the Will Corporation of Rochester, N. Y., from which it may be obtained.

TRAUMATIC HEMOLYSIS AND THE WASSERMANN REACTION

By George Manghill Olson, M.D., Minneapolis, Minn.

THE red blood cells of man and animals are rather fragile in structure, in comparison with the other body cells.

The destruction of red blood cells is evidenced by hemolysis, and is caused by thermal changes, by bacteria, by chemicals, by drying and trauma or rough handling.

The fragility of the red blood cells of various animals varies considerably, the red blood cells of the dog being most fragile and sheep's blood most resistant to mechanical or chemical injury.*

Rous and Turner quoted by Kolmer and Brown* found that human blood was more resistant than sheep's blood. I have found that over half of the specimens of human blood for the Wassermann reaction, when collected from a vein by the syringe method, show marked hemolysis when the serum has separated. The serum is often tinted to a degree resembling port wine. This traumatic hemolysis is due to the force required in discharging the blood from the syringe into the vial, and occurs even when the specimen is placed immediately in the ice box.

Marked traumatic hemolysis also often occurs when specimens of blood are obtained by puncture and squeezing from the finger.

In order to avoid this hemolysis which may yield false positives, specimens of blood for the Wassermann reaction should be obtained from a vein, the blood flowing through the needle into a vial.

Hemolysis of sheep's blood can easily be produced by even moderate trauma when washing with salt solution. Sheep's corpuscles, when placed in salt solution and centrifuged, tend to cling to the bottom of the tube. When the supernatant salt solution is removed and more salt solution added, it requires thorough shaking to remove the corpuscles from the bottom of the tube. This shaking often re-

*Kolmer, J. A., and Brown, C. P.: Am. Jour. Syph., April, 1919.

sults in slight hemolysis, even when perfectly fresh sheep's blood is used. Repeated washings serve only to increase the trauma, so that the fourth or fifth washing may show more hemolysis than the second.

The use of these red blood cells in the Wassermann reaction may result in complete hemolysis in the positive controls, or as a rule in slight hemolysis in the positive controls.

To avoid traumatic hemolysis, I use the following method in washing sheep's corpuscles:

Two or three c.c. of sheep's blood are poured into 50 c.c. centrifuge tubes filled with 0.9 per cent cold salt solution. The tubes are centrifuged at moderate speed. The supernatant salt solution is removed. The tubes are again filled with salt solution. A small bit of cotton is wound around the tip of a wooden applicator. With this cotton tipped applicator the corpuscles are carefully removed from the bottom of the tube and gently stirred. Two washings are sufficient.

SUMMARY

1. Specimens of blood for the Wassermann reaction should be collected from a vein, the blood flowing through the needle into the vial or tube.

2. The syringe method of collecting blood from the vein and the method of collecting blood from the finger should be abandoned.

3. In order to avoid hemolysis in washing sheep's corpuscles, thorough shaking of the centrifuge tubes should be avoided, a uniform suspension can be obtained by gentle stirring with a cotton tipped wooden applicator.

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EDITORIALS

The Functional Pathology of Surgical Shock

BY CONCERTED investigation in experimental medicine in England and in this country, remarkable progress was made during the last two years of the war in the elucidation of the causes of the condition known as shock. There are several varieties of this condition, the two most characteristic of which are surgical shock met with in the operating room and after severe accidents in civic practice, and secondary trench shock met with at the battle front. In every essential particular these two conditions appear to be alike, and a condition apparently identical with them can be produced in laboratory animals by various experimental procedures. It is largely because of the availability of this experimental material that it has been possible to throw so much light on the problem. This but serves once again to illustrate the necessity of animal experimentation in the furtherance of medical and surgical knowledge. Had it not been for the work done on shock in the laboratory by Bayliss, Cannon, Dale, Erlenger, and others, the war might have ended without our being any further advanced in our knowledge of this mysterious and fatal condition. It may be of interest here to review very briefly some of this experimental work.

We shall consider first of all the investigations by Dale,[1] and Laidlaw and Richards[2] on the shock-like condition which is produced by injections of histamine (iminazylethylamine). This substance is derived by removal of the carboxyl group, as CO_2, from histidine, one of the most important of the building stones of the protein molecule. Injected quickly into etherized animals in very minute dosage (1 mg. per kg. body weight) histamine soon causes the arterial blood pressure to fall to the shock level of 30-40 mm. Hg. For a brief period preceding the fall there is a rise in pressure due to constriction of the arterioles, and this constriction persists while the pressure is falling. So far as the obvious vascular changes are concerned, therefore, the condition is strictly comparable with those found in shock—low blood pressure and constricted arterioles. By the time the pressure has fallen to near the shock level the cardiac pulsations disappear from the tracing. The respirations also cease, but if the animal be kept alive by artificial respiration and the thorax opened for inspection of the heart this organ will be observed to be beating quite vigorously, with, however, a pronounced deficiency of blood in the auricles and in the large veins both of the thorax and abdomen. This observation affords positive proof that in this form of shock at least the fundamental cause for the condition is inadequate blood flow to the heart. The question is, what becomes of the blood? Either it must pass out of the blood vessels into the tissues, or the capacity of the former must be increased. Loss of blood itself could scarcely occur short of hemorrhage—of which there is no evidence in histamine shock—but the water with some of the soluble constituents (plasma) might become extravasated, leaving in the vessels blood excessively rich in corpuscles. Such extravasation actually occurs in acute histamine shock, as revealed by measurement either of the concentration of hemoglobin or of the corpuscles, but this in itself can not explain all of the loss in circulating blood, for if the histamine be given slowly (over a period of 20-30 min.) it takes much longer for the shock to become established, and the blood does not show any increase in the percentage of hemoglobin or in the number of corpuscles. In these cases we are driven to conclude that much of the blood must be withdrawn from currency by stagnation in dilated vessels. Direct evidence for this important conclusion has been secured by determination of the volume of circulating blood, by means of the vital red method of Keith, Rowntree and Geraghty,[3] described elsewhere.

Although the oligemia is due in great part to dilatation of the capillaries and venules of the intestine, as can be shown by inspection, it is also partly dependent upon dilatation of vessels elsewhere, since histamine shock can be induced in animals from which all of the intestines have been removed. The vessels of the skeletal muscles are probably the chief extraabdominal vessels affected, for although no dilatation of these can ordinarily be seen in histamine shock, it becomes quite evident in animals which have been transfused before being shocked. The capillaries (and venules) in these areas evidently lose their tone so that they become too roomy for the available blood. As a matter of fact Dale and Richards[2] have shown that histamine abolishes the tone of capillaries at the same time that it increases the permeability of the walls and so permits the plasma to leak through. It is on account of this latter action that histamine when it is rubbed on the scarified skin soon causes the formation of a wheal like that following the lash of a whip.[4]

When histamine is given to unanesthetized animals about ten times as much can be withstood as in those that are anesthetized with ether.[5] At first sight this result might seem to discount the observations on etherized animals, but on the contrary they greatly enhance their importance. They indicate that whereas the normal animal is able to combat the toxic action of histamine, ether greatly depresses this power, an observation which agrees remarkably with the clinical experience that administration of ether is most dangerous in persons who are threatened with shock. The poisoning effect of ether persists for some time after the anesthetic is removed, and it is no doubt dependent upon a toxic action on the endothelium of the capillaries, for it is particularly in such animals that concentration of the blood is evident after histamine. It is of great significance that histamine did not readily produce shock in nitrous oxide anesthesia.

Hemorrhage also greatly predisposes to histamine shock, but in this case the blood is not nearly so concentrated as ordinarily because of the passage of plasma from the tissue spaces into the vessels, which, it will be remembered, is the natural reaction of an animal to hemorrhage alone. The cause of shock in such animals is mainly the opening up of the vessels.

Many bacterial toxins, both when applied to scarified skin and when injected intravenously, have effects very like those of histamine. It is also well known that shock is peculiarly common after injuries in which there has been extensive destruction of tissue. The facts warrant the suggestion that shock may be due to liberation from damaged tissues, particularly the muscles and the viscera of toxic substances acting like histamine. This conforms with the fact that shock is most common when there has been extensive destruction of muscle, or when the liver or intestines are roughly handled. It is possible also that the shock of intestinal obstruction is fundamentally due to absorption into the blood of similar substances from the closed loop of intestine. Whipple and Hooper's discoveries that absorption of a proteose is responsible for the shock-like symptoms of intestinal obstruction are very suggestive in this connection.[6]

But to return to surgical shock. Is it possible that the condition is dependent upon intoxication by histamine-like substances absorbed from greatly damaged tissues? To test this hypothesis Cannon[7] and others have investigated the effects of crushing the muscles of the hind limbs, without external hemorrhage, by blows from a heavy hammer. It was found that an immediate fall in blood pressure occurred, followed by a more gradual decline to the shock level, with a decrease in the CO_2-combining power and a marked concentration of the blood. This result was not due to irritation of afferent nerves, causing excessive stimulation of the vasomotor centers, since it persisted in animals in which all nerves of the limb had been cut; neither was it caused by any local loss of circulating fluid (by dilatation of vessels or extravasation). It was due to the discharge into the circulation of some toxic material, since no shock resulted when the vessels of the damaged limb were clamped. Removal of the clamp some time after the damage resulted in the immediate appearance of the symptoms which could again be caused to disappear somewhat by its reapplication. As to the nature of the toxic material, the first possibility to be considered is that it is unoxidized acid (lactic), which, it is well known accumulates quickly in muscular tissue whenever this is destroyed, or when the circulation through the tissues is greatly

curtailed. As a matter of fact it was found that the CO_2-carrying power of the blood became greatly depressed whenever the toxic material was permitted to enter the circulation by removal of the clamp, and it is well known that there is also a decided depression in the blood carbonates in surgical shock. Acid intoxication can not, however, be the main factor, and for the following reasons: 1. Injections of lactic acid intravenously do not cause shock, neither do they predispose an animal to it. 2. Copious injections of bicarbonate solution do not prevent shock. 3. Extracts of damaged muscle made with isotonic saline do have a shock-like effect, but this is just as great when the lactic acid in the extracts is neutralized with bicarbonate, as when they are unneutralized. Moreover the fall in the blood carbonate does not coincide with, but rather precedes, the development of the shock symptoms. An excess of lactic acid in the blood has been noted in the later stages of many cases of shock (Wiggers and Macleod), but this is a secondary effect, and it is doubtful whether it is the only cause for the depressed CO_2-carrying power of the blood.

In one or two cases the muscles were crushed in unanesthetized cats, with the result that shock did not invariably follow, but this does not invalidate the observations on anesthetized animals; it only shows that, as in histamine poisoning, the anesthetic weakens the resistance. When the normal animals were bled before the crushing operation, shock supervened with certainty.

Taking the results as a whole and comparing them with clinical experience a very strong case is made for the hypothesis that surgical shock is essentially due to intoxication by materials derived from damaged tissue. Shock is particularly common after severe tissue damage; rough handling of the wound greatly aggravates it, whereas rigid care to render the wounded part immobile is a valuable safeguard; the administration of ordinary anesthesia, (ether) to a shock patient is notoriously dangerous, whereas rapid amputation under nitrous oxide often ushers in a steady recovery. All these clinical facts conform admirably with the experimental findings.

With regard to the diagnostic value of measurement of the blood volume, it has been shown by Erlanger, Gasser and Meek[8] that concentration of the blood becomes evident before the shock symptoms are pronounced. This concentration is no doubt a most important factor in causing curtailment of the volume of circulating fluid, not only because of loss of plasma, but also because it causes the corpuscles to become contiguous so that they have a tendency to jam in the capillaries and so lead to a progressively increasing under-nutrition of the tissues and the production of more toxic material.

It remains for us to show that the foregoing conclusions drawn from observations made on laboratory animals are applicable to the clinical condition known as surgical shock. It will then be advantageous to consider the principles which determine successful treatment. The unusual opportunity afforded at the front to study shock has led to a furtherance of our knowledge of its causes, which might have taken many years of investigation in time of peace, and by far the most important contributions have come from those who have been intimately familiar with the experimental as well as the clinical aspect of the problem. N. M. Keith[9] estimated the total volume of circulating blood by the vital

red method and the relative amounts of plasma and corpuscles by measurement of hemoglobin or by means of the hematocrit, and as a result of his investigations has divided the cases of secondary shock into three groups which vary from one another with regard to: 1. The total volume of blood in circulation and (2) the relative amounts of plasma and corpuscles in the blood. The differentiation is not only of great prognostic value, but also invaluable as a guide to the proper plan of treatment. In group 1 are the *compensated cases,* in which the blood volume is reduced to not more than 80 per cent of the normal, but in which the plasma is relatively greater, being reduced only to 85 or 90 per cent of the normal. In other words these cases have reacted like cases of hemorrhage, i. e., there has been a migration of fluid from the tissues into the blood. If kept warm and given fluid per rectum, the patients recover. In the second group, called *partially compensated,* the blood volume is reduced to 65-75 per cent, with little, if any, evidence of dilution of plasma (i. e., the plasma is also reduced to 65-75 per cent). Treatment by transfusion either with blood (citrated blood by Robertson's method,[10] or with gum solutions (*vide infra*) is necessary and in most cases, if the proper technic is followed in the transfusion, recovery is likely. It is important, however, that the plasma volume be measured a few hours after the transfusion to see whether the desired reaction, namely, a migration of fluid into the plasma, has set in. If not so, a second transfusion is indicated. In favorable cases the plasma volume increases more rapidly than that of total blood, and *pari passu* the arterial blood pressure rises.

In the third or *uncompensated group*—the blood volume is below 65 per cent and the blood is more concentrated than normal, i. e., there is relatively a greater decrease of plasma. Treatment must be energetic in these cases, but the prognosis is unfavorable because the transfused fluid readily leaves the vessels, causing the lungs and tissues to become edematous.

With regard to the rationale of the transfusions, it is clear that the added fluid makes good the blood that is lost by stagnation, etc., and so tends to maintain in the circulation a normal pressure for a sufficient time to enable the organism to destroy the toxic bodies. If the shock condition has existed for some time, so that the nerve centers are paralyzed, the injections are of no avail. Since many cases of shock in man have also suffered considerably from loss of blood, it is often difficult to decide whether shock really exists apart from the effects of hemorrhage, the cardinal symptoms of the two conditions being very much alike. The test is afforded by examination of the total blood and plasma volume, and by the reaction to transfusion. After hemorrhage alone there is great migration of plasma into the blood, making this very dilute, and transfusion has immediately beneficial results. In shock there is no migration of fluid into the blood, indeed the reverse is usually the case, and transfusion does not always succeed in re-establishing normal conditions.

Finally, with regard to the composition of the transfusion fluid, should this be human blood, or can a reliable substitute be found in saline solutions containing gum? There is much diversity of opinion over this question. Keith sums up by stating that there does not appear to be any decided advantage in blood over gum solutions, although the immediate restoration of natural color to the pa-

tient, which occurs with blood but not with gum solutions, may make the former appear to be the more satisfactory treatment.

Much painstaking work has been done by Erlanger and Gasser[8] to determine the exact conditions for success in using gum solutions. As their criterion for successful treatment, they did not merely see whether the blood pressure was restored, but they allowed the animals to recover from the effects of the anesthetic and then watched them to see whether they became restored to normal. Many animals might appear to be recovering, but nevertheless succumb within 24 hours. These workers point out that strong gum solutions owe their efficacy to the fact that they slowly attract water into the blood from the tissues, and once attracted the water remains in the vessels. Hypertonic solutions of crystalloids on the other hand, quickly attract water, but this is not retained long. These workers, therefore, devised the scheme of combining the two factors, and they found that success depended on how this was attempted. In the shock produced by partial clamping of the vena cava about one-half of the animals died within 48 hours. Neither weak gum (6 per cent) and weak alkali (2 per cent) given in large amount (12 c.c. per kg.) nor strong gum (25 per cent) in strong alkali (5 per cent) given in smaller dosage (5 c.c. per kg.) decreased the above mortality; but if strong gum (25 per cent) were given along with strong glucose solutions (18 per cent) at the rate of 5 c.c. per kg. an hour, many more animals survived. The alkali was chosen to furnish the crystalloid, in many of the experiments, so that it might incidentally combat any existing acidosis. We have already seen, however, that there is no reason to believe that acidosis is an important factor in shock. Two precautions are necessary to success in using the gum solutions, first they must be properly prepared, and second they must not be injected so rapidly that their high viscidity would slow the circulation and so embarrass the heart's action.

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—*J. J. R. M.*

The Cause of Yellow Fever

A REVIEW of the history of bacteriology, showing as it does, within the short interval of fifty years, the discovery of the causative agents of so many diseases can not but make one most optimistic regarding future developments in that field. We are today intimately acquainted with the organisms causing several diseases whose etiology ten years ago, was quite unknown. Later de-

velopments have been especially concerned with the so-called filter passers, or filterable viruses, such as the globoid bodies found in poliomyelitis and the organisms of the spirochetal class. Further developments in the study of these filter passers may be expected to enlighten us on many more of the common infectious diseases.

Yellow fever has been described until the present time as a disease due to an unknown ultramicroscopic virus, capable of passing through a porcelain filter. Noguchi announces the discovery of an organism in the blood and tissue of cases of yellow fever, allied morphologically and biologically to the parasites of the spirochete class, and which, when transferred experimentally into guinea pigs, produces a disease, similar both clinically and pathologically to yellow fever in man.

This organism he designates as leptospira icteroides, and describes as "an extremely delicate filament measuring 4 to 9 microns in length and 0.2 of a micron in width, along the middle portion. It tapers gradually toward the extremities, which end in immeasurably thin sharp points. The entire filament is not smooth but is minutely wound at short and regular intervals, the length of each section measuring about 0.25 microns. The windings are so placed as to form a zigzag line by alternate change of direction of each consecutive portion at an angle of 90 degrees. The organism is unrecognizable by translucent light, but becomes quite visible under a properly adjusted dark-field illumination. It possesses active motility consisting in vibration, rotation, rapid bipolar progression and sometimes of twisting of parts of the filament. When it encounters a semisolid substance it penetrates the latter by a boring motion, and while passing through the body assumes a serpentine aspect with few undulations, the elementary windings undergoing no modification.

"The organism manifests remarkedly flexibility to almost any angle while changing its course of progression in a semisolid medium. In a fluid medium it has fewer and quite characteristic movements. One end is usually bent in the form of a graceful hook, and, while rapidly rotating, the organism proceeds in the direction of the straight end, the hooked end apparently serving as a sort of rear propeller. When extricating itself from an entanglement, the same hooked end seems to act like the front propeller of an airplane. Many specimens are seen with both ends hooked, the organism then rotating in a stationary position unless one hook is larger and more powerful as a propeller than the other. The rapid rotation makes the organism appear like a chain of minute dots. From the dynamic point of view the portions which include the several windings from the extremities, represent the motor apparatus of the organism."

Staining of the organism is difficult, and best results are obtained by Giemsa's method after osmic acid fixation.

The parasite is not an anaerobe, and requires a certain amount of oxygen for growth. Blood serum is essential. The organism is highly sensitive to the reaction of the medium, and best growth occurs in a solution slightly alkaline to litmus. Bacterial contamination usually rapidly destroys the parasite.

Best results were obtained by cultivation in media consisting of one part blood serum and three parts Ringer's solution. The organism grows slowly and produces no macroscopic change in the culture media.

It is highly virulent, has been known to kill a guinea pig in a dose of .000001 c.c. and retains its virulence for at least thirty-seven days. After four months, the organism had lost some of its virulence, but this was brought back by animal passage.

It has occasionally been found in the blood and tissues of individuals and animals sick with the disease, usually being present in the blood two to three days after infection. In the moribund the organisms were decidedly less frequent both in the blood and the organs.

The description of this organism as being present in cases of clinically characteristic yellow fever, and as being the probable cause thereof must be accepted as the most satisfactory explanation of the etiology that has so far been produced. This germ, isolated in pure culture has been shown to produce a similar disease when reinoculated into laboratory animals, and from them it can be again obtained in pure culture.

Further proof will depend upon immunologic reactions. These have been developed in Noguchi's work, but have evidently not been completed. Guinea pigs inoculated with the blood of yellow fever patients, which survived, and were subsequently reinoculated with organ emulsions obtained from experimentally infected animals as a rule did not die from the second injection, whereas controls did die. We must notice here, that pure cultures were not used.

Intraperitoneal inoculation of convalescent patients' serum together with cultures of the organism was found to result in a positive Pfeiffer phenomenon in 83 per cent of cases. Moreover these pigs had fewer deaths after this inoculation than the controls. The latter showed no Pfeiffer phenomenon.

Noguchi was able to reproduce the experimental disease in guinea pigs from bites of Stegomyia mosquitos which had over 12 days previously fed on yellow fever patients, or over 8 days previously fed on animals sick with the experimental disease.

The close resemblance of the leptospira icteroides, in all respects, to the leptospira icterohemorrhagica of infectious jaundice, and the fact that this latter disease is found in the locality where the yellow fever studies were made, call for considerable discretion and convincing proof before their identity has been denied. The rather close clinical resemblance between the two diseases must also be remembered. This renders the work particularly difficult, with both diseases occurring in the same community.

Noguchi studied the leptospira found in several rats captured in the vicinity, and found them to be those of infectious jaundice. He further differentiated them from the icteroides by their immunologic reactions.

The sera of rabbits immunized against the leptospira found in rats at Guayaquil agglutinated the leptospira icterohemorrhagica and protected guinea pigs when inoculated with a mixture of this serum and the infectious jaundice organism, while they had little or no agglutinating action on the leptospira icteroides or protective action when inoculated together with it.

Guinea pigs inoculated with the killed rat organism proved resistant to infection with leptospira icterohemorrhagica. Noguchi does not state that pigs inoculated with the killed rat organism were not protected against the leptospira icteroides.

Finally, we must remember that this organism was demonstrated only in a small proportion of the cases studied.

Further studies and further refinements of methods will give us additional immunologic data from which to judge, and may enable the observers to find the parasite in a higher proportion of the cases. We shall also expect to see further reports on experiments with Stegomyia calopus mosquitos as the carrier of this organism or some phase of its life cycle.

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—*W. T. V.*

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*Agglutination of B. Anthracis**

NOBLE has investigated this matter. Previous experimenters have reached diverse and even contradictory conclusions. Sobernheim in a review of the subject states that the agglutinating action on anthrax bacilli may be demonstrated both microscopically and macroscopically, but on account of the immobility of the bacilli and their tendency to clump spontaneously, the test is unsatisfactory; that some serums give distinct reactions in high dilutions, while others from highly immunized animals fail wholly. Sacwtschenko states that horse serum, whether from normal or immunized animals always agglutinates the anthrax bacillus, while dog serum never does. Cavins claims to have obtained serum from vaccinated animals, which agglutinate in high dilutions, from 1-50000 and even as high as 1-500000.

After studying the literature Noble comes to the conclusion that the difference in results has been due to failure to secure homogeneous emulsions and this he claims to have done by the following method: The bacillus is transplanted daily for ten days on plain agar at 42.5 degrees until a sporeless and vigorous growth is obtained. It is then grown on the same medium and at the same temperature in quart whiskey flasks for 12 hours. These growths are washed off with physiologic salt solution to which 0.5 per cent formalin has been added (about 100 c.c. to a flask). These suspensions are shaken in a mechanical shaker for forty-eight hours and allowed to stand for several days, during which time they are tested for sterility. Then a shaking for twenty-four hours follows. After standing overnight, the supernatant part is filtered several times through four thicknesses of sterile cheesecloth. The suspensions are now diluted with physiologic salt solution containing 0.5 per cent formalin to a point corresponding to a suspension of B. typhosus containing 2000 million bacteria per c.c. Suspensions of b. anthracis thus prepared are perfectly

*Jour. of Immunology, iv, 105.

homogeneous and stand at 37 degrees for at least forty-eight hours without spontaneous clumping and are suitable for agglutination tests.

These suspensions were tested with the serums from thirteen horses which had been immunized first with vaccines and then with increasing doses of the virulent organism and contrasted with similar tests with the serums of seven untreated horses. The tubes with the serums of the normal animals ran from 1-80 to 1-200, while with the serums of the immunized horses it ran from 1-6400 to 1-20000.

In order to secure homogeneous suspensions the cultures must be sporeless and free from old organisms, not more than eighteen hours old. The suspensions must be well shaken, the clumps allowed to settle, and carefully strained.

—*V. C. V.*

Classification of Spiral Organisms (Spirochete, etc.).

IN CONJUNCTION with the study of an organism of the spirochete type as the probable cause of yellow fever, it is of interest to review briefly the later classifications of this family of parasites. The group has been known for so short a time, and new forms are being added so constantly, that numerous changes will undoubtedly be necessary before a standard classification will be attained.

The spirochete originally described was a large organism, more closely allied to the vegetable or protozoal form of life, nonparasitic, living in fresh water. We agree with Noguchi and others who prefer to keep that name for that particular type of organism. Two other organisms belonging to the family of spirals are included in the classification, but they are much more closely allied both in size and habitat to the original spirochete as described. Of these, the latter cristispira, is parasitic in the alimentary canals of fish, while the saprospira is purely saprophytic. All other genera have pathogenic forms. The three first genera have then been included, as a help in the comparative differentiation of the pathogenic members of the group.

Spironema is the word used to designate the organisms more familiarly known as spirilla, while treponema is used for the generic name of organisms similar to the Treponema pallidum. This name was preferred even by Schaudin himself, to the more usual designation of spirochete.

The accompanying table of classification following Noguchi, is the best classification of the spiral organisms, with which we are acquainted.

It has many drawbacks. Former standards required that organisms presenting transverse division be classed as bacteria, while those dividing longitudinally be called protozoa. Such a standard would require the designation of the original spirochete the saprospira and critispira as bacteria, which they obviously are not. Again the presence of an undulatory membrane, or crista, would place the cristispira also among the protozoa. Finally the possibility of longitudinal fission would throw the spironemata and treponemata also into this class.

CLASSIFICATION OF SPIRAL ORGANISMS

GENUS		SPIROCHAETA ("COIL-HAIR")	SAPROSPIRA ("PUTRID-COIL")	CRISTISPIRA ("CRESTED COIL")	SPIRONEMA ("COIL-THREAD")	TREPONEMA ("TO TURN, THREAD")	LEPTOSPIRA ("FINE-COIL")
Measurements	Length	Microns—100 to 150	100 to 120	45 to 90	8 to 16	6 to 14	7 to 9 or 14
	Diameter	—Microns 0.5 to 0.75		1.0 to 1.5	0.35 to 0.5	0.25 to 0.3 Cylindrical	0.25 to 0.3
	Width of spiral	2				1.0 Regular Rigid	0.45 0.5
	Length of spiral	1.5 Regular				0.8 to 1.0 Very constant	0.3 Regular
Ends	Shape	Blunt		Blunt	Pointed	Pointed	Pointed
Waves	In addition to finer spirals	Several large, inconstant, irregular	Large, inconstant, shallow, irregular. 3 to 5 in number	Two to five or more Large, irregular. shallow.	Large, wavy spirals, usually five	One or more slight undulating wave.	One or more gently throughout entire length. One or both ends circularly hooked when in liquid media. Serpentine in semisolid media. Extremely flexible.
Axial Filaments		Present (flexible elastic)	Absent	Absent	Present (?)	Doubtful	Absent
Chambered Structure		Absent	Present	Present	Absent	Absent	Absent
Membrane		Absent	Present (flexible elastic)	Present (flexible elastic)	Delicate flexible double contoured	Doubtful	Absent
Terminal	Finely spiral filament	Absent	Absent	Absent	Present	Present	Not recognized
Flagella		Absent	Absent	Absent	Absent	Absent	Absent
End	Highly motile end portion	Absent	Absent	Absent	Absent	Absent	Well developed in last 6-8 spirals
Crista	Undulating membrane	Absent	Absent	Present ridge-like membrane spirally about body.	Absent	Absent	Absent
Division	Character	Transverse	Tranverse	Transverse	Transverse possibly also longitudinal	Transverse possibly also longitudinal.	Transverse
Habitat		Fresh or Marine water	Foramminiferous sand	Parasitic in alimentary canals of fish	Numerous pathogenic and nonpathogenic varieties.	Two pathogenic and several harmless parasites.	Two pathogenic and one possibly nonpathogenic varieties.
Action of Chemicals	Trypsin Digestion	Axial filament resistant		Membrane resistant Crista and chambers disappear.		Resists for many days.	
	Bile Salts 10%	Becomes shadowy, pale but is not dissolved.		Crista destroyed Body not attached.	Complete disintegration.	Disintegration complete	Easily dissolved
	Saponin 10%	Lives 30 minutes, later becomes shadowy, pale but not dissolved.		Crista becomes fibrillar and then indistinct. Body not affected	Immobilized in thirty minutes. Broken up in a few hours.	Broken up in time.	Completely resistant.

Just as there are dogs and horses and cattle, so in the microscopic forms of life, there are numerous varieties which can not be compressed by an artificial nomenclature into one species. Disease may be caused by bacteria, by protozoa, by organisms corresponding to the spiral species, which we prefer to consider apart from both of the preceding, by vegetable parasites, and by organisms of the globoid body type. And the list is probably not yet completed.

—*W. T. V.*

The Teaching of Preventive Medicine in Medical Schools

THE national board of medical examiners which examines graduates of only our best medical schools after at least one year of hospital service finds practically all the candidates who come before the board lacking grossly in their knowledge of preventive medicine. This is because of the fact that they have had no adequate instruction along this line. The whole curriculum of our medical schools is devoted to curative medicine, and no adequate instruction is given in the prevention of disease. Early in the war the Surgeon General authorized

one of his assistants to select and place in each camp and with each division a competent epidemiologist. A few medical men who had served as health officers of cities were found to be qualified for this position, but the rank and file of the medical profession did not know the A B C of preventive medicine. Very few knew how to compute an annual death rate, and the ignorance concerning the methods of tracing infection and preventing its spread was appalling. It was necessary to turn over the whole question of water supply and its purification to sanitary engineers, and, with a few exceptions mentioned above, members of the engineering profession supplied the best epidemiologists. We are not complaining; we are simply stating facts. It is probably right that the medical school should devote its chief attention to curative medicine. But we must face the facts and determine whether or not the practice of preventive medicine is to be assumed by some other profession. In most of our medical schools the chair of bacteriology is combined with that of pathology, and all attention is centered on the lesions produced by bacteria, and but little thought given to the control and limitation of infectious diseases. Preventive medicine is coming more and more rapidly to the front. The commissioner of health in the state of Ohio is seeking a competent health officer for each county in the state. Will he be able to find these men in the medical profession, and if found, will they demonstrate their fitness? We must recognize the fact that our medical schools do not fit their students for the practice of preventive medicine, and this must be made a specialty to be acquired by the pursuit of specially arranged courses extending for one or two years after graduation in medicine.

—V. C. V.

Sir William Osler

THE cable brings us the sad news that Sir William Osler died at his home, Oxford, England, December 29. Last November Dr. Osler was stricken with pneumonia, but about the middle of that month it was reported that he was convalescing favorably. Then came more doubtful reports, but these left a ray of hope. On Christmas day he cabled his greetings to his old friends at the Johns Hopkins Hospital and announced that he was making a good fight after an operation for empyema. The cable, however, announces his death.

William Osler was the sixth son of an English clergyman and was born in Canada. He had his education at Trinity College, the University of Toronto and McGill University. After graduation he spent some time in Europe and became a member of the faculty of McGill University. Later he was called to the Professorship of Medicine in the University of Pennsylvania. When the medical school of John Hopkins University was organized about 1880 the board of trustees with great wisdom selected three eminent men to determine the destinies of that school. These three men were Osler, Welsh, and Halsted. This is not the place to dwell upon the great service that these men have rendered to American medicine. We must content ourselves with noting that each became the most potent factor in the scientific development of his specialty.

Dr. Osler represented a distinguished family; distinguished for strength of character, thoroughness in work, and the highest ideals. One of his brothers became a great financier and played an important part in the economic and finan-

cial development of Canada. This brother was knighted in 1912. Another brother became a member of the Supreme Court of Ontario and was noted for his wisdom and justice. A third brother for many years was one of the most distinguished members of the Canadian bar.

Every medical student in this country and Canada who has pursued his studies during the past forty years has had close association, intellectually if not personally, with Doctor Osler. His work on the practice of medicine in the various editions in which it has appeared, has had more influence upon the physicians of this country than any other book. In excellent and classical English he has initiated thousands of young men into the mysteries of disease and pathology. Doctor Osler was a close, keen, and accurate observer. He kept records of all his cases and was able to speak at all times and about all diseases with the certainty of one who had seen for himself. Doctor Osler is dead. This will be said thousands of times and always with the deepest regret. But his spirit and his work will long remain bright and shining lights to guide, not only those who are now living, but those who are to be born in the path of scientific medicine.

Doctor Osler's only son, an officer in the English artillery, was killed in France. The grief which this death caused bore heavily upon the distinguished father, but his home in Oxford was always open to medical men from this country. He assisted young and old medical officers from this side who went over early or late to give their service in the great emergency. His house was a Mecca to which all American medical officers traveled with deepest reverence and where they found a cheerful welcome and an ever ready helping hand.

The world has lost a great man. In Canada, England and the United States his death will be universally mourned. He was known not only to the medical profession, but to the educated world at large.

—V. C V.

Spirochetes

IT is only very recently that we have obtained anything like a satisfactory understanding and a classification of those interesting parasitic microorganisms generally classified under the name of Spirochetes. The very nature of spirochetes, whether animal or vegetable, has been the subject of continued discussion. Originally regarded as plant forms, they were subsequently considered in certain of their examples as animal parasites, but the consensus of opinion today would seem to place them as intermediate between the two, and more in the nature of plant than animal. (Chandler.[1])

The reasons for regarding spirochetes as more like bacteria than protozoa rests on the fact that they have no nucleus, that they divide by transverse division, and show no evidence of a sexual process. The less weighty arguments that have been offered for regarding them as protozoa are that some of their members have a crista-like membrane which suggests the undulating membrane of the trypanosomes, that they stain with difficulty, as do many of the protozoa, and that they frequently require insect intermediate hosts for their transmission.

[1]Chandler, Asa C.: Animal Parasites and Human Disease, 1918, John Wiley & Sons, New York.

Owing to the difficulty in cultivating spirochetes, of studying them in their living condition, and often of staining them in a satisfactory manner, much confusion has arisen as to the varieties that may be legitimately made in these interesting microorganisms. Noguchi[2] recently has suggested the first comprehensive and apparently thoroughly satisfactory classification of spirochetes. He divides them into six different genera—Spirochæta, Saprospira, Cristispira, Spironema, Treponema, and Leptospira. Of these groups the first three are of no particular pathogenic significance.

The first organism of the spirocheta group was the first spirochete to be described and was due to Ehrenberg in 1838. Several species of this genus have since been found in salt and fresh water and are characterizd by their considerable length (100 to 500 micra), by the fact that they have no membrane, that they contain plasmic spirals and volutin granules. These characteristics, as indeed those of the subsequent genera to be mentioned, are far better visualized in Noguchi's admirable diagrammatic representations, than understood by mere descriptive terms.

Members of the saprospira group were described first by Gross in 1911. They are also long forms, though not so long as the spirocheta, and have a chambered structure. They occur in sand.

The cristispira group, also described by Gross (1910), is exemplified in the Cristispira balbiani, an inhabitant of the crystalline style of shell fish.

Numerous members of the next, or spironema genus are of pathogenic significance. They are small, (8 to 16 micra) twisted microorganisms with a permanent spiral filament and include many organisms that are the causative agents of the different forms of recurrent fever in man, formerly referred to as the Spirochetes of Obermeyer, etc. Among them may be mentioned *Spironema recurrentis, S. carteri, S. duttoni* and the like.

The spironema group may be easily separated from the treponema group in typical examples according to Noguchi. The treponemata as first described by Schaudinn (1905) in connection with the causative agent of syphilis are somewhat shorter (6 to 14 micra) than the spironema organisms, and are characterized by pointed ends, a spiral filament and regular and rigid spirals. The causative agent of syphilis (*Treponema pallidum*) and of yaws (*Tr. pertenue*) would be placed in this group, as well as certain nonpathogenic members of the group, like Tr. microdentium and Tr. macrodentium.

Last of all comes the genus newly differentiated by Noguchi (1917) of Leptospira. These organisms have pointed and hooked ends, a corkscrew-like appearance, and differ from other spirochetes in that they are very resistant to the action of saponin. Their locomotion, which is extremely active, differs somewhat in nature from the other varieties. The most important members of this genus that have hitherto been described are the leptospira of ichtero-hemorrhagic fever (L. icterohemorrhagiæ) and the recently described Leptospira icteroides which is regarded by Noguchi as a causative agent in yellow fever.

—*F. P. G.*

[2]Noguchi, H.: Morphological Characteristics and Nomenclature of *Leptospira* (*Spirochaeta*) *Ictero-haemorrhagiae* (Inada and Ido), Jour. Exper. Med., 1918, xxvii, 575.